LINKING THE MAJOR SYSTEM MARKERS FOR TYPICAL BRAIN DEVELOPMENT

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Summary

The transition from childhood to adulthood is accompanied by marked changes in brain structure and function. The changes seen in this time include well-documented trends in grey and white matter structures as well as in the functional networks. These developmental changes are possibly associated with the emergence of most mental health disorders in adolescence. Moreover, a divergence in the prevalence of psychiatric disorders between males and females becomes apparent during adolescence which may suggest a role of puberty and its associated (hormonal) changes. Importantly, sex differences are not only present in the prevalence of disorders but also in many aspects of human brain anatomy and physiology, with women for instance consistently showing higher cerebral perfusion than men. While much is already known about individual aspects of the human brain regarding sex differences and development, the underlying mechanisms remain largely in the dark.

Accumulating evidence exists for the diverse effects of steroid hormones on human brain physiology and anatomy. Another group of highly relevant factors acting on different cerebral systems are neurotransmitters and metabolites, in particular the main excitatory and inhibitory neurotransmitters glutamate and γ-aminobutyric acid (GABA), respectively. However, investigations of the associations between the various factor and their changes during development are so far scarce. Therefore, this thesis aimed to investigate the relationships between a set of selected major system markers of typical human brain development.

In a first study, the link between steroid hormones and cerebral perfusion was investigated, and specifically, the possible role of these hormones as underlying factors for the known sex difference in cerebral perfusion. The results revealed associations between the steroid hormones and cerebral perfusion and they suggest that dehydroepiandrosterone sulfate (DHEAS) in particular may be an important factor accounting for the higher perfusion seen in women compared to men.

In a second study, the link between the developmental changes in the levels of the main neurotransmitters (i.e. GABA and glutamate) and developmental changes in functional connectivity was investigated. The results showed an increase in subcortical and cortical GABA+/glutamate with age and second, they suggest that the developmental decrease in subcortical glutamate/H_2O might mediate an age-related decline in local functional connectivity in the dorsal putamen.

Taken together, these findings advance the knowledge on the intricate interactions between major system markers in typical brain development and they provide further evidence for the contributions of these interactions to the various brain changes seen during the transition from childhood to adulthood. Furthermore, these interactions may consequently be highly relevant in

their role as factors leading to sex differences in the brain, and they may play an important part in the emergence of many psychiatric disorders during adolescence.

Zusammenfassung

Der Übergang von der Kindheit in das Erwachsenenalter ist gekennzeichnet von deutlichen Veränderungen in der Struktur und Funktion des Gehirns. Zu den während dieser Zeit vorkommenden Veränderungen gehören Änderungen in der grauen und weissen Substanz wie auch in der funktionellen Vernetzung. Diese Entwicklungsveränderungen stehen vermutlich in einem Zusammenhang mit dem Erstauftreten vieler psychischer Störungen in der Jugendzeit. Des Weiteren geht während dieser Zeit die Prävalenz vieler psychischer Störungen auseinander zwischen männlichen und weiblichen Jugendlichen. Dies kann ein Hinweis darauf sein, dass die Pubertät und die damit verbundenen (hormonellen) Veränderungen eine Rolle spielen bei der Entstehung psychischer Störungen. Unterschiede zwischen den Geschlechtern kommen nicht nur in der Prävalenz vor, sondern auch in vielen Aspekten der Anatomie und Physiologie des menschlichen Gehirns. So haben Frauen zum Beispiel eine höhere zerebrale Durchblutung als Männer. Während schon vieles bekannt ist in einzelnen Aspekten des menschlichen Gehirns bezüglich Geschlechtsunterschiede und Entwicklung, so sind die zugrundeliegenden Mechanismen noch weitgehend unklar.

Es mehren sich die Belege für diverse Effekte von Steroidhormonen auf die Hirnphysiologie und -anatomie. Eine weitere Gruppe von hochrelevanten Faktoren, die auf verschiedene Hirnsysteme wirken, beinhaltet die Neurotransmitter und Metaboliten, speziell die exzitatorischen und inhibitorischen Hauptneurotransmitter Glutamat und γ-Aminobuttersäure (GABA) . Untersuchungen zu den Zusammenhängen zwischen den verschiedenen Faktoren und ihren Entwicklungsveränderungen sind jedoch rar. Aus diesem Grund hatte die vorgelegte Dissertation das Hauptziel, die Beziehungen zwischen ausgewählten Systemmarkern der typischen Hirnentwicklung des Menschen zu untersuchen.

Die erste Studie untersuchte den Zusammenhang zwischen Steroidhormonen und Hirndurchblutung mit einem besonderen Augenmerk auf die mögliche Rolle dieser Hormone in der Erklärung der bekannten Geschlechtsunterschiede in der Hirndurchblutung. In der Tat wurde ein Zusammenhang zwischen Steroidhormonen einerseits und der Hirndurchblutung andererseits gefunden. Die Resultate legen zudem nahe, dass Dehydroepiandrosteronsulfat (DHEAS) ein besonders wichtiger Faktor zu sein scheint in der Erklärung des Umstandes, dass Frauen im Vergleich zu Männern eine höhere Hirndurchblutung zeigen.

Die zweite Studie untersuchte den Zusammenhang zwischen Entwicklungsveränderungen in der Konzentration der Hauptneurotransmitter (d.h. GABA und Glutamat) einerseits und Entwicklungsveränderungen in der funktionellen Vernetzung andererseits. Die Resultate zeigten erstens eine mit der Entwicklung einhergehende Zunahme des Verhältnisses GABA+/Glutamat in einer subkortikalen und einer kortikalen Region. Zweitens legen die Resultate nahe, dass eine

mit der Entwicklung einhergehende Abnahme von subkortikalem Glutamat/H_2O einen entwicklungsbedingten Rückgang der lokalen funktionellen Vernetzung im dorsalen Putamen vermittelt.

Zusammenfassend erweitern diese Ergebnisse das Wissen um die komplexen Interaktionen zwischen wichtigen Systemmarkern typischer Hirnentwicklung und sie liefern weitere Belege dafür, dass diese Interaktionen zu den verschiedensten Entwicklungsveränderungen des menschlichen Gehirns beitragen können. Ferner können diese Interaktionen in der Folge hochrelevant sein in ihrer Rolle als Faktoren, die zu Geschlechtsunterschieden im Gehirn führen, und es ist sehr gut denkbar, dass sie bei der Entstehung vieler psychischer Erkrankungen im Jugendalter mitwirken.

1 General Introduction

Human brain and behaviour undergo marked changes from childhood to adulthood. Every parent can testify to clear and noticeable changes in the behaviour of their children within the time of childhood and adolescence. Parents can experience and observe their children developing from a highly dependent newborn from the day of birth through childhood with many different challenging phases and steps of development, through the possibly demanding time of puberty and adolescence, to finally a grown, mature, and fully independent adult. Possibly, many parents ask themselves during this time what might be going on in the brains of their kids. How is their brains' structure changing? How are the brain regions and the network between them formed and refined? What happens in the brain's chemistry during this time? Does something happen differently in the brains of boys and the brains of girls? How are all these things linked with each other?

Most, if not all, these questions have been addressed in some fashion within the field of human neuroscience in the last few decades. A myriad of studies have investigated and examined a large variety of aspects of the development of the human brain by use of an extensive number of different research methods. We thus know much about many different facets of human brain development from childhood to adulthood, e.g. about developmental changes in brain structure, physiology, networks, neurotransmitters, and also about gender or sex differences in these different aspects. In addition, efforts have been made to examine the relations between changes in these different systems. Despite the large body of research in this field, much remains unknown, especially with regard to the associations between the different aspects of human brain development. This thesis thus had the aim to further elucidate the relationships between major system markers of typical brain development. Specifically, this thesis focused on investigating the links between two major system markers each. Firstly, linking steroid hormones and cerebral perfusion, especially to examine the possible role of steroid hormones as underlying factors for the known higher cerebral perfusion found in women. And secondly, linking developmental changes in the main excitatory and inhibitory neurotransmitters to concomitant changes in functional connectivity. Before addressing the specific questions and methods used in this thesis, a short general overview is given of the current state of knowledge on typical brain development, followed by a more detailed view on the two specific topics.

1.1 Typical human brain development

Regarding whole brain volume, a review of magnetic resonance imaging (MRI) studies showed a wave of growth occurring during childhood and adolescence, which levels off and at age 13 a gradual decrease in volume sets in (Hedman, van Haren, Schnack, Kahn, & Hulshoff Pol, 2012). Another wave of growth, or at least a period of stability, may follow in young adulthood, and after age 35, the whole brain volume begins to decrease (Hedman et al., 2012). On a less global level, grey matter (GM) and white matter (WM) measures show distinct courses during development. In longitudinal studies, GM volume has been reported to follow an inverted u-shaped trajectory in most brain regions as well as in whole brain volume, showing a preadolescent increase followed by a postadolescent decrease (Giedd et al., 1999; Lenroot et al., 2007). GM maturation (expressed by GM density) demonstrated distinct trajectories in different brain regions in a longitudinal study (Gogtay et al., 2004). Specifically, the findings demonstrated that primary sensorimotor cortices develop first, and the remainder of the cortex mostly matures in a "back-to-front" direction. Thus, phylogenetically newer regions mature later than older ones, and, only after lower-order areas have matured, the higher-order association areas, which integrate the lower functions, fully develop. Studies on WM volumes quite consistently demonstrated developmental increases, both globally and regionally (Lebel, Walker, Leemans, Phillips, & Beaulieu, 2008; Paus, 2010).

The most popular explanations for the structural developmental changes in grey and white matter have been synaptic pruning and myelination (cf. e.g. Paus, Keshavan, & Giedd, 2008). Regional differences have been demonstrated in the time courses of both synaptogenesis and synaptic pruning, i.e. the elimination of synaptic contacts that are not included in neuronal circuits (Huttenlocher & Dabholkar, 1997, but cf. Purves, White, & Riddle, 1996 for a critical view on this so-called 'neural Darwinism'). Again, primary sensory areas exhibited earlier and faster developmental changes than prefrontal areas. It is thus tempting to postulate that decreases in cortical thickness might reflect a loss of synapses. Additionally, a reduction in the number of synapses is likely accompanied by a corresponding decrease in metabolic demand and thus by a reduced number of glial cells (Paus et al., 2008). Consequently, this could account for a decline in regional GM volume and/or thickness (Paus et al., 2008). However, it is also possible that cortical thinning could simply reflect increased myelination in the WM structures near to the border to GM (Jernigan, Baare, Stiles, & Madsen, 2011; Paus et al., 2008), leading to a change from "grey" signal of these unmyelinated fibres that become more "white" with increasing deposition of myelin. However, this view has also been disputed by a study investigating the relationship between GM, WM, and diffusion tensor imaging (DTI) measures in a large developmental subject group (Tamnes et al., 2010). Their findings indicated that cortical thinning could not be explained by WM maturation in underlying WM regions as measured by volumetry or DTI.

In summary, it is clear that dynamic biological changes in the brain tissues continue throughout childhood and adolescence (Jernigan et al., 2011). It remains unclear, however, to what extent putative factors such as pruning and myelination play a role in the changes in morphology or which other possible factors contribute to the striking developmental changes observed during this time (Jernigan et al., 2011).

1.2 Sex Differences in the Brain and the Potential Role of Steroid Hormones

The transition from childhood to adulthood is not only accompanied by major changes in brain structure and function but also by increasingly larger divergence in these facets between boys and girls (Lenroot & Giedd, 2010), which leads to sex differences still present in adults (Ruigrok et al., 2014). A review by Lenroot & Giedd (2010) showed that the most consistent finding is the apparent larger brain size in males compared to females. Other findings include regional differences in brain morphology, with basal ganglia and limbic structures most frequently showing sex differences, as well as sex-specific neurodevelopmental trajectories with both total and regional GM volumes peaking earlier in girls and more rapid WM growth in boys.

The emergence of sex differences in the human brain during adolescence represents one argument for the importance of this period. Another is provided by the fact that the peak age of onset for most mental health disorders also falls into this time (Kessler et al., 2005). And, importantly, the female:male prevalence ratios of many disorders show a marked switch from approximately equal ratio in prepuberty to a 2:1 female:male prevalence ratio after puberty (Paus et al., 2008). This is especially evident in depression (Angold & Costello, 2006). Additional evidence for the importance of puberty comes from studies showing that this divergence in prevalence appeared only after a certain pubertal status had been reached and that this phenomenon was better predicted by pubertal status than by chronological age (Paus et al., 2008).

The precise factors underlying sex differences in the brain, in the prevalence of disorders, and in their emergence during adolescence are not yet known. The most intuitive culprits are arguably the "raging hormones" associated with puberty (Paus et al., 2008). Indeed, some consistency has been reported between regions showing structural sexual dimorphism and those with high amounts of sex steroid receptors in animals in early development (Goldstein et al., 2001; Lenroot & Giedd, 2010). Furthermore, sex steroid levels have been demonstrated to be differentially related to GM and WM changes in pubertal boys and girls (Peper, Hulshoff Pol, Crone, & van Honk, 2011), and to GM in sexually dimorphic regions in adults (Witte, Savli, Holik, Kasper, & Lanzenberger, 2010). Most neurons either have receptors for adrenal and gonadal hormones, which thus might directly affect neuronal structure and function (Paus et al., 2008). Alternatively, they might be indirectly influenced by the steroid hormones via (other) neurotransmitters such as dopamine and serotonin since both dopamine and serotonin nuclei have been shown to be sensitive to (sex) steroids and these nuclei project diffusely to wide areas of the brain (Lenroot & Giedd, 2010). Virtually all basic mechanisms of the remodelling of the adolescent brain (e.g. neurogenesis and synaptogenesis as well as apoptosis and synaptic pruning) are known to be influenced by steroid hormones (Sisk & Zehr, 2005). Sisk & Zehr (2005) accordingly noted that "it is important to recognize the onset of puberty not as a gonadal event, but

rather as a brain event" (p. 164). However, our understanding of the relationship between hormones and the brain remains poor, last but not least owed to the complex and reciprocal nature of the interaction (Paus et al., 2008).

In comparison to the abundance of research conducted on sex differences in brain structure, so far only little focus has been on sex differences in cerebral perfusion, and their underlying factors. Women have consistently been reported to show higher cerebral perfusion than men in studies using a variety of different techniques (Devous, Stokely, Chehabi, & Bonte, 1986; Esposito, Van Horn, Weinberger, & Berman, 1996; Gur & Gur, 1990; Yinan Liu et al., 2012; Parkes, Rashid, Chard, & Tofts, 2004; Podreka et al., 1989). Interestingly, in a study investigating sex differences in cerebral perfusion in a group of healthy children, girls older than 12 years of age exhibited higher regional brain perfusion than boys, while this sex difference was not evident in boys and girls younger than 12 years of age (Taki et al., 2011b). Since the onset of puberty occurs around 12 years (Sun et al., 2002), puberty and its associated changes (i.e. in sex steroid hormones) seems likely to underlie not only the structural sex differences described above, but also the sex differences in cerebral perfusion observed in adolescents and adults. Additionally, studies have reported that this sex difference is not present anymore between older men and women (Gur & Gur, 1990), which could indicate a role of menopause and the associated changes in sex steroid concentrations. In support of this hypothesis, sex steroid hormones have been shown to influence vascular reactivity (for a review, cf. Krause, Duckles, & Pelligrino, 2006). Specifically, oestrogens enhance production or sensitivity to vasodilatory factors while androgens show the opposite effect. Besides the sex steroids as obvious candidates, other steroid hormones may also play a role in differentially affecting perfusion in men and women. The adrenal hormones cortisol and dehydroepiandrosterone sulfate (DHEAS) have also been shown to be associated with perfusion (Akishita et al., 2008; Murialdo et al., 2000; Wang, Rao, et al., 2005; Wang et al., 2007). Moreover, DHEA(S) (referring to both DHEAS and its non-sulphated precursor DHEA) has been reported to be higher in men than in women (Mazat et al., 2001; Orentreich, Brind, Rizer, & Vogelman, 1984; Šulcová, Hill, Hampl, & Starka, 1997; Tannenbaum, Barrett-Connor, Laughlin, & Platt, 2004) and importantly, this sex difference in DHEA(S) is most significant starting around puberty (Elmlinger, Kühnel, & Ranke, 2002; Peretti & Forest, 1978; Šulcová et al., 1997).

1.3 Developmental Changes in Neurotransmitters and Functional Connectivity

Besides hormones as underlying factors influencing human brain anatomy and physiology, an arguably even more obvious group of agents acting on the brain are neurotransmitters, especially the main excitatory neurotransmitter glutamate and the main inhibitory neurotransmitter γ-Aminobutyric acid (GABA). Magnetic resonance spectroscopy (MRS) allows the *in vivo* detection and quantification of glutamate, GABA, and of a range of other brain metabolites or neurotransmitters. The possible roles of a variety of these metabolites in the brain have been extensively discussed elsewhere (e.g. Rae, 2013). Reports on age-related changes in different metabolites have varied considerably (Duncan, Wiebking, & Northoff, 2014; Haga, Khor, Farrall, & Wardlaw, 2009). Changes in creatine and in N-acetylaspartate (NAA) with age have been reported relatively consistently and these findings are important to consider with regard to confound control in the Study B included in this thesis. Specifically, both creatine and NAA are often used as references for the quantification of other metabolites or neurotransmitters (cf. also Chapter 4.2.2) including the quantification of the neurotransmitters of interest for Study B, i.e. glutamate and GABA.

1.3.1 Developmental changes in the levels of the main excitatory and inhibitory neurotransmitters

Glutamate and GABA are the key constituents of the excitation/inhibition balance, which primarily determines neuronal activity (Duncan et al., 2014; Isaacson & Scanziani, 2011; Lauritzen, Mathiesen, Schaefer, & Thomsen, 2012; Logothetis, 2008). In adults, glutamate has relatively consistently been reported to decrease with age (Chang, Jiang, & Ernst, 2009; Hädel, Wirth, Rapp, Gallinat, & Schubert, 2013; Kaiser, Schuff, Cashdollar, & Weiner, 2005; Schubert, Gallinat, Seifert, & Rinneberg, 2004). The few reports of glutamate changes in studies including children and adolescents showed increases of glutamate with age, however, these differences were most prominent in neonates, and the glutamate concentration seemed to reach a plateau in childhood with no further increases (Blüml et al., 2013; Degnan et al., 2014; Raininko & Mattsson, 2010). For GABA, the picture seems less clear, with some studies reporting age-related decrease of GABA during adulthood (Gao et al., 2013; Grachev, Swarnkar, Szeverenyi, Ramachandran, & Apkarian, 2001), while others found no relation between age and GABA (Goddard et al., 2001; Goto et al., 2010). The only study so far on GABA including healthy adolescents reported an increase of GABA with age (Silveri et al., 2013), and one study including only children found an increase with age (Gaetz et al., 2014), while another found no significant correlation (Edden, Crocetti, Zhu, Gilbert, & Mostofsky, 2012). Generally, the results seemed to depend on the region investigated since many studies reported significant age-related changes in some regions measured but not in others (e.g. Chang et al., 2009; Gaetz et al., 2014; Silveri et al., 2013).

1.3.2 Developmental changes in neural networks and connectivity

Associations between regional neurotransmitter levels and brain activity as well as cerebral perfusion have been demonstrated in adults (Duncan et al., 2014). Most studies, however, investigated these links in very basic sensory processing tasks or during rest (Duncan et al., 2014). Importantly, only few studies examined the link between neurotransmitters and connectivity measures, and so far no study related neurotransmitter changes to functional connectivity in a subject group including children and adolescents in addition to adults. It is important to understand how interregional interactions develop from childhood to adulthood and not only how the structure and function within individual brain region changes (Vogel, Power, Petersen, & Schlaggar, 2010). The human brain is a complex integrative network of different brain areas, which each have their own function but are at the same time continuously sharing information and consequently forming a very efficient network of structurally and functionally linked brain regions (van den Heuvel & Hulshoff Pol, 2010). Thus, besides activity in secluded brain regions, human brain function importantly includes intricate interactions between those regions and these interactions exhibit remarkable changes from childhood to adulthood (see below).

Since the second study of this thesis included an investigation of developmental changes in functional connectivity measured with functional MRI (fMRI), the two most important broad themes on this topic are described here. First, anatomical segregation and functional integration of networks have emerged as two general principles of developmental changes in functional connectivity (Power, Fair, Schlaggar, & Petersen, 2010; Rubia, 2013; Tau & Peterson, 2009; Vogel et al., 2010). That is, while the brain networks of children appear to be organised largely by anatomical proximity, adult networks seem to follow an organisation by their functional roles, i.e. they are integrated into their respective networks (functional integration), and are more distributed across the brain (i.e. anatomically segregated). And second, this trend of progressive segregation and integration with development is accompanied by a decline in short-range (i.e. local) connectivity strength between anatomically adjacent but functionally distinct networks in children, and by a concomitant rise in long-range (i.e. distal) connectivity strength between the distributed regions that comprise the adult networks (Power et al., 2010; Rubia, 2013; Tau & Peterson, 2009; Vogel et al., 2010).

1.3.3 Potential links between neurotransmitters and connectivity

Similarly to the discussion of putative mechanisms for developmental changes in brain structure (cf. Chapter 1.1), synaptic pruning and myelination have been suggested to underlie the changes in functional connectivity (Power et al., 2010; Vogel et al., 2010). Importantly for Study B, it has been argued that the functional significance of synaptic pruning during adolescence likely involves adjustments of the excitation/inhibition balance both on individual neu-

rons and within networks, which consequently might be related to developmental changes in functional connectivity (Selemon, 2013). Thus, investigations of the relationship between developmental changes in the main excitatory and inhibitory neurotransmitters (glutamate and GABA, respectively) and concomitant changes in functional connectivity are of high interest. Regarding these neurotransmitters separately, glutamate has been shown to play a role in synchronizing neuronal networks (Rodriguez, Sabate, Rodriguez-Sabate, & Morales, 2013), and GABA has been reported to be involved in nearly all key developmental steps in the cortex as well as in the experience-dependent refinement of local circuits (Di Cristo, 2007).

1.4 Acquisition of the data for this thesis

The data for Studies A and B included in this thesis were acquired in a multimodal MR protocol including structural, functional, perfusion, and spectroscopy measurements. Additionally, blood and saliva samples were collected to obtain hormone values. In total, 86 healthy subjects were included in this project with ages ranging from 8.1 to 53.3 years. Since in each Study A and B the links between different selected system markers were investigated, and since the full acquisition of the complete dataset was not possible for every single subject, the final subject numbers included in Studies A and B differed from this grand total (cf. Chapters 2.3.1 and 3.3.1 for the study-specific numbers and reasons for exclusion of single subjects).

1.5 Methods to Study the Link between Cerebral Perfusion and Steroid Hormone Concentrations

Before the description of the methods for Study B (i.e. MRS and functional connectivity analyses), the methods available to examine the link between cerebral perfusion and steroid hormones (i.e. the aim of Study A) are described in this section. Cerebral perfusion or blood flow can be measured with various techniques such as single-photon emission computed tomography (SPECT, e.g. Devous et al., 1986) or positron emission tomography (PET, e.g. Esposito et al., 1996). A clear disadvantage of these methods lies in the fact that they are highly invasive due to the necessity of injection of gamma-emitting radionuclides as tracers. As an alternative approach, arterial spin labelling (ASL) MR-perfusion imaging methods can provide non-invasive, quantitative measurements of local and global cerebral blood flow. Analogous to PET measurements, arterial blood water is "labelled" in ASL techniques, but in contrast to PET, which uses ^{15}O labelled water as a tracer, this labelling procedure involves radiofrequency (RF) pulses that "decays" with T1 relaxation rather than with a radioactive decay rate for ^{15}O (Detre, Rao, Wang, Chen, & Wang, 2012). Typically, ASL data are obtained from successive pairwise subtractions between images that are acquired with the "magnetically labelling" RF pulses and the control images that are not labelled. Similar image appearance and blood flow values have been demonstrated for ASL MRI and ^{15}O-PET measurements (Detre et al., 2012).

The possibility to obtain quantitative measures with ASL measurements of cerebral perfusion represents an advantage over other MR-based techniques using blood oxygenation level-dependent (BOLD) contrast since BOLD signal changes are usually expressed as a relative percentage in signal change and are based on a statistical model and significance level (Detre et al., 2012). In addition, the BOLD signal stems from a complex interaction between several physiological variables that accompany neural processes and include cerebral blood flow (CBF), but also cerebral blood volume and cerebral oxygenation metabolic rate (Detre et al., 2012). However, ASL measurements are not without limitations of their own. The signal-to-noise ratio (SNR) for ASL imaging is substantially lower than for BOLD and the acquisition time for ASL images is relatively lengthy since it includes about one second each for label, delay, and image acquisition (Aguirre & Detre, 2012). Consequently, to improve sensitivity quite thick slices are being used, and to shorten acquisition time fast read-out imaging techniques (i.e. echo-planar imaging) are used, with the disadvantage of distortions in regions of high static susceptibility gradients that degrade image quality (Aguirre & Detre, 2012). Several other technical challenges exist in ASL imaging, for example a sensitivity to arterial transit time of the label which led to the now routine employment of a postlabelling delay (Detre et al., 2012). Experimental and theoretical studies to improve the accuracy perfusion quantification by ASL have been conducted (Detre et al., 2012) and consequently, today's estimation methods take into account various pa-

rameters (e.g. capillary water permeability, magnetisation transfer effects, arterial transit time, T1, labelling efficiency) and use background-suppression to enhance the temporal SNR (for further details on the ASL method applied in this thesis, cf. Chapter 2.3).

Since the aim of Study A was to investigate the link between cerebral perfusion and steroid hormones, blood and saliva samples were collected from the subjects. Serum concentrations of oestradiol, testosterone, and DHEAS as well as saliva concentrations of cortisol were measured via immunoassay (for further details, cf. Chapter 2.3). For all these hormones, some methodological aspects need to be taken into consideration (for a further discussion, cf. Chapter 4.1.2). Diurnal variations in concentration have been shown for all hormones measured in this study. The distinct circadian rhythm of cortisol levels has been shown consistently (Chan & Debono, 2010), and variations in the levels throughout the day have also been reported for oestradiol (Bao et al., 2003), testosterone (Brambilla, Matsumoto, Araujo, & McKinlay, 2009), and to a lesser extent also for DHEA (Hucklebridge, Hussain, Evans, & Clow, 2005). For study A, the blood sampling was thus limited to the afternoon and the saliva sampling (to obtain the cortisol values) to 16.00h, which both was achieved with fair success. While these measures were taken to control for possible timing confounds, they proved to be too limited for the cortisol acquisition specifically and did not allow drawing sufficiently reliable conclusions regarding the effects of cortisol (for suggestions of approaches optimising these aspects, cf. Chapter 4.1.2).

Naturally, when investigating hormonal effects in premenopausal women, one has to keep in mind that the sex steroid hormone levels in women undergo changes within the menstrual cycle (cf. e.g. Stricker et al., 2006). This issue was addressed by keeping a recording the onset of the last period in the female subjects to estimate their current cycle phase. Another aspect to consider is the fact that many young women use hormonal contraceptives that affect their sex steroid hormonal levels. Thus, many young women had to be excluded from certain analyses and female participants who were not under hormonal contraceptives were specifically recruited. Lastly, difficulties arise from the fact that the concentration levels of the sex steroid hormones are very low in prepubertal children (Zec et al., 2012) and that the analytical sensitivity of the assays presently used are often too low, i.e. the children's hormone levels do not reach the lower limit of detection (Rahhal, Fuqua, & Lee, 2008). This problem also occurred in the present study, and thus it was not possible to determine the levels of oestradiol and testosterone in most of the participating children, resulting in the inclusion of only healthy adult subjects in Study A.

1.6 Methods to Study the Link between Neurotransmitters and Brain Networks

Magnetic resonance spectroscopy (MRS) provides a powerful technique to non-invasively detect and quantify chemical compounds in the human body *in vivo* (Bottomley, Edelstein, Foster, & Adams, 1985; Brawn & Vincent, 2014; Mullins et al., 2014; Puts & Edden, 2012). The very brief description of this methodology given here follows mostly Brawn & Vincent (2014) and Puts & Edden (2012). Radiofrequency signals arising from hydrogen nuclear spins within neurochemicals are detected by MRS. These signals have chemically specific frequencies that are determined by the electronic and chemical environment (i.e. the molecular structure of the neurochemical) in which the hydrogen spins are embedded. Consequently, the MRS signals that arise from neurochemicals with different molecular structure are separated in the MR spectrum along chemical lines. This is known as the chemical shift dimension and is reported in ppm (parts per million of the proton frequency). The second dimension of a spectrum contains the signal amplitudes, which are roughly proportional to the substance amount and thereby provide a measure of their concentrations. In single-voxel spectroscopy as used in this study, spectra can be obtained from predefined regions of interest (or "voxel"). Only metabolite concentrations in the millimolar range are detectable with MRS since the signal amplitudes of molecules with lower concentrations are not sufficient to exceed the inevitable measurement noise. This inherently poor sensitivity is the reason why some highly interesting neurotransmitters such as dopamine, which e.g. is present in concentrations in the micromolar range, cannot be measured with MRS. Fortunately, the concentrations of the two major excitatory and inhibitory neurotransmitters are high enough: glutamate is present in the brain in relatively high concentrations (~12 mM) and GABA narrowly exceeds this lower detection limit (~1 mM; Rae, 2013).

The SNR depends heavily on the homogeneity of the magnetic field within the voxel of interest and thus a preparatory step, known as shimming, is performed to optimise the field homogeneity before the actual measurements. Even in case of good shimming, the dispersion of the different peaks along the chemical shift axis is limited. As a result, the peaks of the signals of the different neurochemicals often overlap and signals of more highly concentrated neurochemicals obscure the signals of less highly concentrated ones. The signals of the two neurochemicals of interest in this thesis, glutamate and GABA, both overlap with other signals. Although the glutamate signal overlaps with the glutamine signal to some extent, at higher field strengths (i.e. 3 T as in this study) the separation of these two signals is feasible (Degnan et al., 2014). For GABA measurements, a difference-edited technique called MEGAPRESS (Mescher, Merkle, Kirsch, Garwood, & Gruetter, 1998) has become the most widely used technique (Mullins et al., 2014). This technique utilises another physical property of nuclear magnetic resonances, the so-called J-coupling, i.e. the coupling of resonances through electrons in chemical bonds. Very briefly, editing pulses at specific frequencies (e.g. at 1.9 ppm for GABA) are added, which selectively affects

the GABA spins at 3 ppm due to the J-coupling while spins of most other neurochemicals are not affected. Subtraction of edited from the non-edited spectra then results in the edited GABA signal. Due to the inherently low SNR of many MRS measures (especially GABA, see above), relatively large voxel sizes are required to ensure adequate signal quality and thereby voxels on the order or 3 x 3 x 3 cm3 are commonly used. Other challenges and limitations exist regarding the MRS method generally and the MEGAPRESS technique specifically (Mullins et al., 2014; Puts & Edden, 2012). For details on how these challenges were met in this thesis, cf. Chapter 3.3, and for a further discussion, cf. Chapter 4.2.5).

The investigation of the developmental trajectories of GABA and glutamate was one of the aims of Study B. The second aim was to examine the relationship between these neurotransmitters and developmental changes in functional connectivity. Functional connectivity has been defined as the temporal correlation between neurophysiological (functional) measurements made in different brain regions (Friston, Frith, Liddle, & Frackowiak, 1993) or, more generally, as the statistical dependencies among remote neurophysiological events (Friston, 2011). Functional connectivity analyses thus examine the temporal coherence of brain function in different brain regions and allow the identification of spatial patterns of coherent BOLD activity (Fox & Raichle, 2007). It is important to note that functional connectivity analyses do not provide information regarding the directionality or the causality of the interregional coherence or of its relationship with other measures such as neurotransmitter levels (Paus et al., 2008). While beyond of the scope of this thesis, investigations of directed and causal relationships between brain regions are feasible by means of analyses of effective connectivity, for example with dynamic causal modeling (Friston, 2011).

Methods to analyse functional connectivity can be roughly placed into two groups: model-free and model-dependent methods, which both have their strengths and limitations (Fox & Raichle, 2007; Hoff, Van Den Heuvel, Benders, Kersbergen, & de Vries, 2013; van den Heuvel & Hulshoff Pol, 2010). One of the most often used model-free methods is the independent component analysis (ICA) approach (e.g. Beckmann, DeLuca, Devlin, & Smith, 2005). A priori hypothesis are not necessary for this technique, which is designed to search for general patterns of connectivity over the whole brain, or, more precisely, for spatial sources of signals that are maximally independent from each other (Hoff et al., 2013; van den Heuvel & Hulshoff Pol, 2010). The interpretation of the resulting independent component maps is often perceived as more difficult compared with the interpretation of the correlation maps obtained by model-dependent methods such as seed-based functional connectivity analyses (Hoff et al., 2013; van den Heuvel & Hulshoff Pol, 2010). Seed-based approaches examine functional connections by correlating the time-series of an a priori defined region of interest (ROI or seed) with the time-series of other regions (Hoff et al., 2013; van den Heuvel & Hulshoff Pol, 2010). While the results are thus limited to the

connections of a predefined ROI and obviously depend on the choice of this region, the simplicity of the analysis and the relative straight forwardness of the results form a strong advantage of these seed-dependent methods (Hoff et al., 2013; van den Heuvel & Hulshoff Pol, 2010). Even though the hypothesis-free ICA method and the hypothesis-driven seed-based method are very different in their methodology, the functional networks found by the two methods correspond fairly well (Rosazza & Minati, 2011). As mentioned above, the second aim of Study B was to examine the relationship between developmental changes in neurotransmitters levels and the concomitant changes in functional connectivity. Since the single-voxel MRS technique used in this study provided measures of GABA and glutamate in a predefined region (subcortical area), the seed-based approach for analysing functional connectivity was used in Study B and the anatomical region most substantially contributing to the MRS voxel (the left putamen) was chosen as the seed region for the correlation analyses. For further details on the method applied and the fMRI dataset chosen for Study B, cf. Chapters 3.3.6 and 3.3.3, and for a discussion of implications of these choices, cf. Chapter 4.2.6).

1.7 General Aims and Hypotheses

This thesis had the aim to investigate the links between a set of selected major system markers for typical brain development. For Study A, the goal was to examine the potential link between cerebral perfusion and steroid hormone concentrations, in light of the fact that sex differences in cerebral perfusion are consistently reported but the underlying factors are poorly understood. An association between these two fundamental features of human physiology seems likely because all hormones investigated in this thesis have been shown to affect perfusion to some degree. Moreover, the physiological concentrations of all the steroid hormones investigated in Study A (i.e. oestradiol, testosterone, and DHEAS) have been shown to differ between men and women. However, so far no study has investigated the role of these steroid hormones as possible underlying factors for the sex difference in cerebral perfusion in the same sample of healthy subjects. We hypothesised that the individual hormone levels would be differentially associated with both global and regional cerebral perfusion, which would support their role as modulators of cerebral perfusion.

The general aim of Study B was to investigate the link between developmental changes in the levels of the main neurotransmitters (i.e. GABA and glutamate) and developmental changes in functional connectivity. Since very little is known so far about age-related changes in these neurotransmitters in children and adolescents, the first goal of Study B was to answer the question of how GABA and glutamate develop between late childhood to middle adulthood. To answer this question, GABA and glutamate values were obtained in two regions: in a subcortical (basal ganglia) region in a group including children, adolescents, and adults; and in a cortical (frontal) region in a group including adolescents and adults. We hypothesised to find a decline of glutamate levels with age and a rise of GABA with age. The second goal of Study B was then to answer the question of how developmental changes in these neurotransmitters are related to the developmental changes in neural networks. We hypothesised that the expected opposing developmental trends of GABA and glutamate would be differentially related to developmental changes in short-range (i.e. local) and long-range (i.e. distal) connectivity.

2 Study A

Effects of Steroid Hormones on Sex Differences in Cerebral Perfusion

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2.1 Abstract

Sex differences in the brain appear to play an important role in the prevalence and progression of various neuropsychiatric disorders, but to date little is known about the cerebral mechanisms underlying these apparent differences. One widely reported finding is that women demonstrate higher cerebral perfusion than men, but the underlying cause of this difference in perfusion is not known. This study investigated the putative role of steroid hormones such as estradiol, testosterone, and dehydroepiandrosterone sulfate (DHEAS) as underlying factors influencing cerebral perfusion We acquired arterial spin labelling perfusion images of 36 healthy adult subjects (16 men, 20 women). Analyses on average whole brain perfusion levels included a multiple regression analysis to test for the relative impact of each hormone on the global perfusion. Additionally, voxel-based analyses were performed to investigate the sex difference in regional perfusion as the correlations between local perfusion and serum estradiol, testosterone, and DHEAS concentrations. Our results replicated the known sex difference in perfusion, with women showing significantly higher global and regional perfusion. For the global perfusion, DHEAS was the only significant predictor amongst the steroid hormones, showing a strong negative correlation with cerebral perfusion. The voxel-based analyses revealed modest sex-dependent correlations between local perfusion and testosterone, in addition to a strong modulatory effect of DHEAS in cortical, subcortical, and cerebellar regions. We conclude that DHEAS in particular may play an important role as an underlying factor driving the apparent difference in cerebral perfusion between men and women.

2.2 Introduction

Sex differences in the brain appear to play an important role in the prevalence and progression of various neuropsychiatric disorders, as well as in learning, emotion perception, and treatment response (American Psychiatric Association, 1994; Cosgrove, Mazure, & Staley, 2007; Hofer et al., 2006). While early childhood disorders like autism and attention-deficit/hyperactivity disorder are more prevalent in males, anxiety and depression are more prevalent in females (American Psychiatric Association, 1994), and differences in the age of onset for schizophrenia have also been reported between men and women (Häfner, Behrens, Vry, & Gattaz, 1991). However, to date little is known about the cerebral mechanisms underlying these apparent differences, despite an increasing body of knowledge about differences in brain structure, function, and morphology between the sexes.

One of the most widely reported findings with regard to baseline brain physiology in men and women is that of an increased rate of perfusion or cerebral blood flow in women. Sex differences in cerebral perfusion have been observed using various techniques including single-photon emission computed tomography (SPECT), positron emission tomography (PET), Xenon-enhanced computed tomography, and arterial spin labelling (ASL) (Devous et al., 1986; Esposito et al., 1996; Gur & Gur, 1990; Yinan Liu et al., 2012; Parkes et al., 2004), both on a global level (Devous et al., 1986; Esposito et al., 1996; Gur & Gur, 1990; Parkes et al., 2004; Podreka et al., 1989) and locally in posterior cingulate cortex, precuneus, and thalamus (Yinan Liu et al., 2012; Taki et al., 2011b). In addition to demonstrating higher perfusion during rest, women have also been reported to show higher perfusion during cognitive activity (Cosgrove et al., 2007). However, the underlying cause of these sex differences remains unclear and the factors modulating this sex difference in perfusion are poorly understood.

One contributing factor for the reported sex differences in perfusion may lie in the combinatory modulation of different steroid hormones (including sex hormones), since these hormones are known to influence the vascular response and to differ between men and women. Specifically, estradiol, testosterone, and dehydroepiandrosterone sulfate (DHEAS) are thought to represent potential modulators of perfusion. Estrogens enhance production or sensitivity to vasodilatory factors (for a review, see Krause et al., 2006), and have been shown to be positively related to cerebral blood flow (CBF) or perfusion in studies applying techniques such as Doppler ultrasound (Nevo, Soustiel, & Thaler, 2007), SPECT (Kaya, Şahin, Köken, Köse, & Cevrioglu, 2008), and PET (Maki & Resnick, 2000). Testosterone, on the other hand, exerts vasoconstrictive effects (Krause et al., 2006), and testosterone supplementation has been reported to decrease CBF in postmenopausal women (Penotti et al., 2001). In men, the local metabolism of testosterone into estradiol via aromatase (Simpson, 2003) might influence the relationship of circulating

testosterone and perfusion to a significant degree. This mechanism may underlie the finding of an increase in CBF in hypogonadal men (Azad, Pitale, Barnes, & Friedman, 2003).

Sex differences are not only present in the sex steroids estradiol and testosterone, but also in DHEAS, which is a precursor of sex steroids. Most studies reported higher levels of DHEAS in men than in women (Mazat et al., 2001; Orentreich et al., 1984; Šulcová et al., 1997; Tannenbaum et al., 2004), while others reported no significant sex differences in DHEAS (Behringer, Hohmann, Stevens, Weltring, & Deschner, 2012; Carlson, Sherwin, & Chertkow, 1999). Nevertheless, a multitude of studies have shown the wide range of functions of DHEAS and its non-sulfated precursor DHEA (collectively referred to as "DHEA(S)") in human physiology, cardiovascular diseases, and brain function and diseases (for reviews, see Dong & Zheng, 2012; Maninger, Wolkowitz, Reus, Epel, & Mellon, 2009; Savineau, Marthan, & Dumas de la Roque, 2013; Tchernof & Labrie, 2004; Traish, Kang, Saad, & Guay, 2011). A few studies reported positive associations between flow-mediated vasodilation of brachial artery and DHEA(S) in postmenopausal women (Akishita et al., 2008; Williams et al., 2004), while others found no effect (Silvestri et al., 2005). One study found a positive correlation between hippocampal perfusion measured with SPECT and DHEAS in patients with Alzheimer's disease but not in controls (Murialdo et al., 2000). The role of DHEAS as an underlying factor in the sex difference in cerebral perfusion therefore remains unclear.

In this study, we investigate whether steroid hormone concentrations are linked to cerebral perfusion, and specifically whether hormone concentrations may explain the previously reported sex differences in perfusion. To our knowledge, no studies have yet investigated the relationships between serum sex hormones, DHEAS and cerebral perfusion in the same healthy volunteers. We use non-invasive ASL, which provides a quantitative measure of tissue perfusion, in contrast to the relative blood oxygenation level dependent response measured by functional magnetic resonance imaging (fMRI). The benefits and the wide range of possible applications of ASL have been demonstrated in a large body of studies in basic and clinical neuroscience (for a review, see Detre et al., 2012). Based on the known sex differences in perfusion and hormone levels, we hypothesized that perfusion correlates positively with estradiol as both are higher in women but negatively with testosterone, which is lower in women. Although DHEAS is also lower in women, we hypothesized that it is positively related to perfusion as found in previous SPECT and ultrasound studies (see above).

2.3 Methods

2.3.1 Subjects

The subject group consisted of 44 adult volunteers (20 males), recruited by local advertisement. Subjects were excluded due to comorbid disorders affecting perfusion (n = 2), caffeine intake shortly before the measurement (n = 1), and technical problems with the ASL acquisition (n = 5). Perfusion data were acquired from the remaining 36 subjects (16 males) and entered into the analysis of the sex difference in perfusion (see Table 2-1 for demographics). Additionally, blood samples were collected for hormone assay (see below) for n = 35 subjects (15 males). Subjects reported no history of neurological or psychiatric illness, illegal substance abuse, or use of psychotropic medication. All subjects gave written informed consent and refrained from caffeine, alcohol, and nicotine for 4 hours (2.5 hours for nicotine in one subject) before the experiment. The study was approved by the Ethics Committee of the Canton of Zurich, Switzerland

2.3.2 Hormone concentration acquisition

Serum concentrations of estradiol, testosterone, and DHEAS were measured on an Elecsys 2010 using commercial Electro-Chemi-Luminescence Immuno-Assays (Roche Diagnostics, Rotkreuz, Switzerland) with coefficients of variations of 8.4 % (256 pmol/L), 4.9 % (7.4 nmol/L), and 4.7 % (2.3 µmol/L), respectively.

Data were collected as part of a larger study investigating age-related cerebral changes of psychophysiological markers from childhood to adulthood. Perfusion data and blood samples were collected during the same measurement session. All measurements were performed in the afternoon or early evening. Hormone data from two female participants were incomplete (n = 1 female with missing estradiol value, n = 1 female with missing DHEAS and estradiol). Regression analyses between hormones and perfusion were performed both for the full group of subjects including all available hormone data and for a subset of participants excluding subjects taking hormonal contraceptives (n = 8 females) or medication affecting testosterone (n = 1 male). For all analyses, there was no difference in age between men and women (see Table 2-1).

Table 2-1: Group demographics and hormone values for the voxel based analyses.

	Men				Women				
Analysis Variable	*n*§	Mean (*SD*)	Median	Range	*n*§	Mean (*SD*)	Median	Range	*p**
Sex difference in perfusion									
Age [years]	16	33.7 (9.9)	32.4	21.4-50.6	20	30.3 (8.6)	27.3	21.0-48.4	.36[b]
Voxel-based correlation between perfusion and sex steroids									
Age [years]	14	33.3 (10.5)	29.1	21.4-50.6	12	33.5 (9.7)	29.6	21.8-48.4	.96[a]
Estradiol [pmol/L]	14	99.73 (40.60)	91.75	59.38-203.90	12	332.70 (160.35)	331.30	103.10-587.80	< .001[a]
Testosterone [nmol/L]	14	17.12 (4.72)	17.29	8.10-25.39	12	0.92 (0.45)	1.04	0.21-1.49	< .001[a]
Voxel-based correlation between perfusion and DHEAS									
Age [years]	15	33.6 (10.2)	30	21.4-50.6	19	30.5 (8.8)	27.5	21.0-48.4	.49[b]
DHEAS [µmol/L]	15	7.78 (4.34)	7.25	2.15-14.43	19	5.40 (2.55)	5.21	0.90-9.91	.07[a]

DHEAS, dehydroepiandrosterone sulfate. All median hormone values were within the reference range (for serum estradiol in men: 93-276 pmol/L, in women: 110-2750 pmol/L; for serum testosterone in men: 7.6-31 nmol/L, in women: 0.2-1.8 nmol/L; for serum DHEAS in men: 1.2-13 µmol/L, in women: 1.0-9.2 µmol/L). **p*-value of comparison between men and women. [a]two-tailed t-test. [b]Wilcoxon rank sum test. § *n* differed for different analyses due to drop-outs (see Methods).

2.3.3 MRI-data acquisition

MR imaging studies were performed with a 3.0 T GE HD.xt whole-body MRI scanner (GE Healthcare, Milwaukee, WI, USA), using an 8-channel receive-only head coil and a body transmit coil. Cerebral perfusion images were collected during an eyes-closed resting condition with a background-suppressed, pulsed continuous arterial spin labelling (pCASL) sequence, using a 3D stack of spirals fast spin echo readout (Dai, Garcia, de Bazelaire, & Alsop, 2008). Thirty-two axial slices were collected with a repetition time (TR) of 5.5 s and an echo time (TE) of 25 ms, a slice thickness (ST) of 4 mm, a field of view (FOV) of 24 cm, 3 Nex, and a nominal in-plane resolution of 1.9 × 1.9 mm^2, and a total scan time of 5 min 17 s. A post-labelling delay of 1.5 s was used to reduce errors from transit time effects (Alsop & Detre, 1996). Structural images were obtained with a 3D T1-weighted gradient echo pulse sequence (number of slices = 172, ST = 1.0 mm, TR = 9.94 ms, TE = 2.948 ms, inversion time = 600 ms, FOV = 256 mm × 192 mm, flip angle = 8°, matrix = 256 × 192, reconstructed voxel resolution: 1 × 1 × 1 mm). The participants were provided with earplugs.

2.3.4 MRI preprocessing

The perfusion images were quantified using the model proposed by Alsop and Detre (1996), with additional terms included to represent the finite labelling duration (Wang, Zhang, et al., 2005) and to correct for incomplete recovery of the magnetisation in the reference image due to the saturation applied t_{sat} (2,000 ms) before imaging. The perfusion was calculated according to the following equation (Alsop & Detre, 1996; Järnum et al., 2010):

$$f = \frac{\lambda}{2\alpha T_{1b}\left(1 - e^{-\frac{\tau}{T_{1b}}}\right)} \frac{S_{ctrl} - S_{lbl}\left(1 - e^{-\frac{t_{sat}}{T_{1g}}}\right)}{S_{ref}} e^{\frac{w}{T_{1b}}}$$

where f is the perfusion (in ml/min/100 ml), $S_{ctrl} - S_{lbl}$ is the difference image (control-label), and S_{ref} is a proton-density weighted reference image. λ is the blood brain partition coefficient (0.9), α is the inversion efficiency, T_{1b} is the T_1 of blood (1,600 ms), T_{1g} is the T_1 of grey matter (1,200 ms), w is the post-labelling delay (1.5 s), and τ is the labelling duration (1.5 s). The labelling efficiency is given by the product of the pCASL labelling efficiency (0.95) and an additional efficiency factor, which incorporates the loss of efficiency from the background suppression (0.75). This equation includes an additional term to correct for incomplete recovery of the magnetisation in the reference image due to a saturation pulse applied t_{sat} (2,000 ms) before imaging (Järnum et al., 2010). The model assumes that the labelled spins remain primarily in the

microvasculature rather than exchanging with tissue water, so the T_1 of blood is used for quantification (Järnum et al., 2010; Wang, Zhang, et al., 2005).

The perfusion images for each subject were normalised to a custom perfusion template in the Montreal Neurological Institute (MNI) space using the flirt algorithm in FSL (fsl.fmrib.ox.ac.uk/fsl/) with the correlation ratio as the cost function. For later statistical analyses, a study-specific template was generated from the normalised perfusion images of 35 healthy adult subjects by first concatenating the images in time and subsequently calculating a mean image across time using the fslmerge and fslmaths utilities from FSL. Finally, the Brain Extraction Tool from FSL was applied to mask this mean image.

2.3.5 Statistical analyses

Statistical analyses of the non-imaging data were performed using MATLAB and Statistics Toolbox Release 2012b (The MathWorks, Inc., Natick, Massachusetts, United States), and IBM® SPSS® Statistics, Version 20. In cases of non-normally distributed data (tested with Lilliefors test), a non-parametric Wilcoxon rank sum test was used (see Table 2-1).

Whole brain perfusion values were extracted to examine differences in global perfusion between men and women and to examine the effects of each hormone on the whole brain perfusion. Specifically, a whole brain grey matter (GM) mask was derived from the AAL atlas (Tzourio-Mazoyer et al., 2002) and registered to the CASL images for each subject in native space, using the flirt algorithm in FSL (fsl.fmrib.ox.ac.uk/fsl/), with the correlation ratio as the cost function. The individual subject's perfusion image was then masked with this GM image using fslmaths, and the mean perfusion signal from this masked image was calculated with fslstats (fsl.fmrib.ox.ac.uk/fsl/). A multiple regression model in SPSS (Enter method) was then used to test for the differential effect of each hormone on the perfusion, with whole brain perfusion as the dependent variable and age, estradiol, testosterone, and DHEAS levels as independent variables.

In all voxel-based analyses on perfusion data, age was included as a covariate or regressor due to the known effect of age on perfusion (e.g. Yinan Liu et al., 2012). Both the sex difference and the correlations between perfusion and hormone levels were tested using the nonparametric permutation-based methods implemented in the Cambridge Brain Analysis (CamBA) software (Chamberlain et al., 2009; Suckling & Bullmore, 2004). The following general linear model was used for all analyses:

$$P = a_0 + a_1 \text{ independent variable} + a_2 \text{ age} + e$$

where P is the perfusion at a particular voxel, a_0 is the mean effect across all subjects, a_1 is the coefficient relating the independent variable vector to perfusion at a particular voxel (i.e. sex in the sex difference analysis and hormone values in the correlation analyses), a_2 is the coefficient for the covariate vector of age, and e is an error term.

This model was regressed at each intra-cerebral voxel onto the observed data to yield a test statistic map of a non-parametric t-value given by the coefficient a_1 divided by its standard error. The model was also regressed 32 times at each voxel after random permutations of the vector coding the respective independent variable (i.e. sex or hormone values) within the subject groups, thus breaking the association between the individual subjects and their individual sex or hormone levels (see Table 2-1 for composition of subject groups). The resulting permutation distributions of a_1, combined over all voxels, were used to derive a preliminary, voxel-level threshold at $p = .05$, which was then applied to observed and permuted maps identically. The sum or "mass" of the resulting suprathreshold voxel statistics was computed for each cluster in both the observed and permuted maps, and these values were ordered to sample the permutation distribution under the null hypothesis of zero difference in perfusion between the sexes or zero correlation between perfusion and hormones within the groups. The mass of each cluster in the observed map was then tested against the critical values obtained from the corresponding permutation distribution. The significance thresholds were corrected for multiple comparisons by setting the number of error clusters accepted to < 1 per image. For the permutation data acquired in the present study, this threshold corresponds to a FWE-corrected p-value of $p < 0.004$.

To determine the degree to which the observed voxel-based hormone correlations may contribute to the observed sex difference, the number of overlapping significant voxels between the sex difference map and the hormone correlation maps was calculated and expressed as a percentage of the voxels in the sex difference map.

In order to assess the impact of sex-based differences in brain structure on the perfusion results, voxel based morphometric (Ashburner & Friston, 2000; Good et al., 2001) differences between men and women were tested with the standard DARTEL pipeline using SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK; www.fil.ion.ucl.ac.uk/spm). T1-weighted images from each participant were first segmented into grey, white, and cerebrospinal fluid images (Ashburner & Friston, 2005). Segmented GM images were “modulated” using non-linear warping procedures that correct for global brain differences and align homologous brain regions into a common space (Ashburner & Friston, 2000; Ilg et al., 2008). The resulting images were then smoothed using a Gaussian kernel with 8 mm full-width at half maximum (Ashburner & Friston, 2000). Between-group analysis was performed on the smoothed images to determine brain voxels where local GM density and volume differed between males and females, after controlling for differences in brain size (using total intracranial volume measures as covariate).

2.4 Results

The results of the hormone assays are given in Table 2-1. As to be expected, estradiol levels were higher in women (M = 332.7 pmol/L, SD = 160.35) than in men (M = 99.73 pmol/L, SD = 40.6, p < .001), testosterone levels were higher in men (M = 17.12 nmol/L, SD = 4.72) than in women (M = 0.92 nmol/L, SD = 0.45, p < .001), and DHEAS levels were higher in men (M = 7.78 µmol/L, SD = 4.34) than in women (M = 5.4 µmol/L, SD = 2.55) on a trend level (p = .07).

As expected from previous reports, both the voxel-based perfusion analysis and the global perfusion analysis demonstrated that perfusion is higher in women. Across the whole brain, perfusion was higher in women (M = 35.97 ml/min/100 ml, SD = 5.37) than in men (M = 30.29 ml/min/100 ml, SD = 6.21, p = .006, see Figure 2-1a). The voxel-based analysis of the sex difference in perfusion revealed an extensive cluster in which women showed higher regional perfusion than men (p = .004, FWE-corrected). This cluster included frontal, parietal, temporal, and occipital regions as well as thalamus, basal ganglia, and cerebellum (see Figure 2-1b). There were no regions in which perfusion was significantly higher in men than in women in the voxel-based analysis.

The multiple regression analysis of the whole brain perfusion data with age, estradiol, testosterone, and DHEAS levels as predictors revealed a significant model (p = .037, R = .545, adjusted R^2 = .197), of which DHEAS emerged as the only significant predictor (standardised β = -.575, p = .012). The standardised β and p-values for the other predictors are as follows: age (standardised β = -.299, p = .173), estradiol (standardised β = .015, p = .936), and testosterone (standardised β = -.099, p = .639). Thus, the regression analysis was repeated with only DHEAS as a predictor, resulting in a significant model (p = .005, adjusted R^2 = .195) and a standardised β = -.468 for DHEAS (see Figure 2-1c). The regression with DHEAS as the only predictor in the subgroup of subjects excluding participants on hormonal contraception or medications affecting testosterone revealed a significant model (p = .031, adjusted R^2 = .145) and a standardised β = -.424 for DHEAS. Two additional single regression analyses for both estradiol and testosterone separately as the only predictors did not reach significance (for estradiol: p = .244, standardised β = .237; for testosterone: p = .435, standardised β = -.160).

The voxel-based correlation analysis between cerebral perfusion and DHEAS levels revealed an extensive cluster showing a negative correlation (p = .004, FWE-corrected). This cluster largely overlapped with the observed sex difference cluster and thus also included frontal, parietal, temporal, and occipital regions as well as thalamus, basal ganglia, and cerebellum (see Figure 2-1d). The calculated overlap between the sex difference map and the DHEAS correlation map was 65 %. This analysis was rerun in two analyses which contained only subjects of one sex and the same DHEAS effect was seen in both men and women separately. Keeping the signifi-

cance level correction to less than one error cluster per image, only the correlation analysis between testosterone and cerebral perfusion in women revealed two small and dispersed negative clusters (p = .003, FWE-corrected). These clusters included bilateral primary and secondary visual cortex extending into precuneus as well as in bilateral thalamus extending into left ventral temporal and cerebellar regions. The ratio between the overlapping significant voxels in both the sex difference and this testosterone correlation map to the whole number of significant voxels in the sex difference map was only 3 %. All other voxel-based correlation analyses (i.e. estradiol in women, estradiol in men, and testosterone in men) did not yield any significant clusters at the chosen significance level (with less than one error cluster per image). At a family-wise error-corrected significance level of $p < .05$, no significant GM differences between men and women were observed.

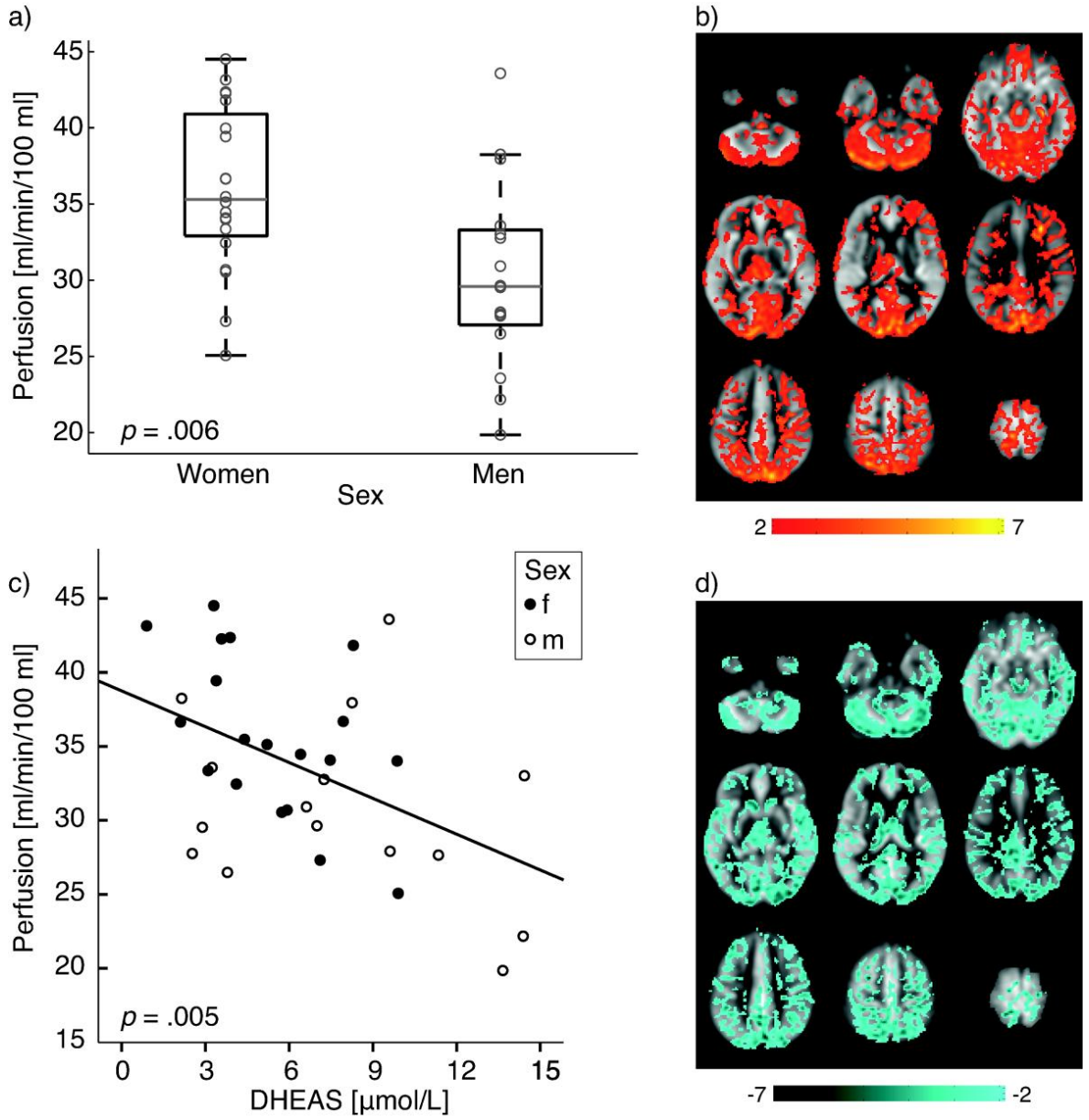

Figure 2-1: Women show higher perfusion than men and DHEAS correlates negatively with perfusion. a) Sex difference in whole brain grey matter perfusion: perfusion is higher in women (M = 35.97 ml/min/100 ml, SD = 5.37) than in men (M = 30.29 ml/min/100 ml, SD = 6.21, p = .006). Single dots represent the subjects' individual values. The horizontal line within the boxes indicate medians, the edges of the boxes are the 25th and 75th percentiles, and the whiskers represent 1.5 times the interquartile range. b) Sex difference (women > men) in regional perfusion: women show higher regional perfusion than men (p = .004, FWE-corrected). c) Simple regression analysis with whole brain perfusion values as the dependent variable and DHEAS as the only predictor: a significant model was found (p = .005, adjusted R^2 = .195) with a standardised β = -.468 for DHEAS. d) DHEAS effects in men and women: DHEAS correlates negatively with regional perfusion in both sexes (p = .004, FWE-corrected). Colour bar in a) and c) denotes a non-parametric t score, given by $a1$/[standard error($a1$)], see methods. Images are shown in neurological orientation. Slices are at MNI z-coordinates -45, -30, -15, 0, 15, 30, 45, 60, 75 (from top left to bottom right).

2.5 Discussion

This study provides the first investigation of the link between steroid hormone levels and perfusion on both a global and regional level. In both the whole brain perfusion analysis and the voxel-based analysis our results replicated the well-known finding that women show higher perfusion than men (Devous et al., 1986; Esposito et al., 1996; Yinan Liu et al., 2012; Taki et al., 2011b). Moreover, we demonstrate for the first time that DHEAS was the only significant predictor of whole brain perfusion amongst the steroid hormones investigated in this study. Additionally, DHEAS showed a strong and widespread negative correlation with cerebral perfusion in the voxel-based analysis, and the corresponding correlation map also overlapped to a large degree (65 %) with the voxel-based sex difference map. While the direct implications of this apparent link between DHEAS and perfusion remain unclear, the higher perfusion in women may partially explain the difference in treatment response observed between men and women (Cosgrove et al., 2007). Investigating the biochemical basis of the reported sex differences and the link between hormones and cerebral perfusion may therefore lend critical insight into the neurobiological basis of various neuropsychiatric disorders, and brain recovery processes.

Our finding of a negative relationship between DHEAS and cerebral perfusion is contrary to our hypothesis, but consistent with studies showing higher DHEAS levels in men (Mazat et al., 2001; Orentreich et al., 1984; Šulcová et al., 1997; Tannenbaum et al., 2004), coupled with the known higher perfusion in women (Devous et al., 1986; Esposito et al., 1996; Yinan Liu et al., 2012; Parkes et al., 2004; Taki et al., 2011b). Additionally, the sex difference in DHEAS is most significant starting around puberty (Elmlinger et al., 2002; Peretti & Forest, 1978; Šulcová et al., 1997), and sex differences in cerebral perfusion have been reported to become significant only in boys and girls older than 12 years of age (Taki et al., 2011b). Broadly, these results suggest that DHEAS may play a strong modulatory role in explaining the sex difference in cerebral perfusion reported previously.

Sex differences have also been reported in cerebral autoregulation in healthy subjects (Deegan et al., 2010). The effects of DHEAS on endothelial function, blood flow (Traish et al., 2011), and its relationship with cerebral perfusion found in this study may point to a role for DHEAS in cerebral autoregulation. While the precise mechanism underlying the autoregulation of cerebral perfusion is not yet fully understood, DHEAS may play a differential role in cerebral autoregulation in men and women, for example due to genetic influences varying between the sexes (see discussion in Tannenbaum et al., 2004). Future studies examining both vascular flow and perfusion following DHEAS supplementation may be able to elucidate further the role of DHEAS in blood flow regulation in men and women.

Interestingly, in postmenopausal women, Akishita et al. (2008) also observed DHEAS as the only significant predictor of flow-mediated vasodilation (FMD) in several multiple regression models including plasma estradiol, testosterone, and cortisol levels as well as age and coronary risk factors as predictors. However, in their study DHEAS was positively associated with FMD. While our finding of a negative relationship of DHEAS and perfusion supports a role of DHEAS in the sex difference in cerebral perfusion, it also seems to contradict the more frequently reported beneficial effects of DHEAS on a different parameters related to blood flow. The reason for these seemingly contradictory results may lie in the differences in the parameters and/or subject populations investigated and in the different methods applied in other studies. For example, a peripheral measure of blood flow in the arm of postmenopausal women was used in other studies, of which some found a positive association between DHEAS and blood flow (Williams et al., 2004), while others did not (Silvestri et al., 2005). In contrast to these non-cerebral and less direct measurements of perfusion, Murialdo et al. (2000) reported a positive correlation between DHEAS levels and hippocampal SPECT findings in patients with Alzheimer's disease but not in controls. Since DHEA(S) has been shown to be involved in numerous physiological functions including endothelial function and blood flow but also including body composition, insulin sensitivity, and cardiovascular disease risk (Traish et al., 2011), the effects of DHEA(S) might present differently in patient groups or older subjects compared to healthy and younger subjects. Maninger et al. (2009) discussed the scarcity of evidence for beneficial effects of DHEA(S) treatment in healthy subjects and argued that benefit from such treatments may be more likely observed in medically or neuropsychiatrically ill patients. Thus, while our results may seem surprising in the light of previous literature, the mechanisms of action of DHEA(S) may differ depending on the subject group investigated, and results may depend on the investigational methods. The precise underlying mechanisms and their differences remain unclear and warrant further study. However, our observation of a negative relationship between DHEAS and cerebral perfusion is consistent with the higher perfusion observed in women and the higher DHEAS levels typically observed in men.

Our other hypotheses regarding the association between cerebral perfusion and estradiol and testosterone were partly confirmed. As hypothesised, cerebral perfusion correlated negatively with testosterone in women. In men, however, no significant correlation between perfusion and testosterone was found. Although more modest, the negative correlation of testosterone with perfusion in women suggests a partial role of this hormone in accounting for sex differences in cerebral perfusion, at least in women. The results of the sex steroid analyses in men, however, do not imply a strong modulatory role of these hormones on cerebral perfusion or a predominant role as an underlying factor for the sex differences in perfusion. These results might also reflect the absence of a direct association between circulating levels of testosterone

and estradiol and their local concentration in the brain (Simpson, 2003). Thus, apart from DHEAS, the circulating levels of steroid hormones investigated in this study seem less likely to represent direct or primary modulators of perfusion and major factors underlying the sex difference in cerebral perfusion.

Circulating levels of estradiol, testosterone, and DHEAS may not completely reflect their concentrations in the brain because these steroid hormones are not only secreted by endocrine glands (i.e. ovaries, testes, adrenals), but also synthesised de novo in neuronal tissue from their respective precursor hormones. It has been estimated that this local synthesis in peripheral tissue from inactive adrenal precursors might be as high as 30 to 50 % for the total androgens in men and up to 75 % for estrogens in premenopausal women (Labrie et al., 1998). Thus, the intracellular levels in the brain may not translate into parallel changes in circulating levels of these hormones (Labrie, Bélanger, Cusan, Gomez, & Candas, 1997). Labrie et al. (1997) suggested that future studies should additionally investigate the levels of the derivates of the hormones of interest since these might be the most reliable estimate of the total androgen pool. Future studies in healthy human subjects additionally investigating these derivates in combination with other known vasomodulatory factors like nitric oxide (Attwell et al., 2010; Krause et al., 2006; Toda, Ayajiki, & Okamura, 2009) may help to elucidate further the modulatory influence of the different steroid hormones on perfusion and their role as factors explaining the sex difference in cerebral perfusion.

2.5.1 Limitations

Given the higher perfusion values in GM relative to white matter, structural differences in brain volume between men and women could confound the assessment of perfusion differences. However, in our sample no significant differences in GM volume were observed with a corrected significance threshold. Therefore, the results of the present study are unlikely to be driven by differences in GM between men and women.

DHEAS was selected as a measurement target in preference to DHEA on the basis of its diurnal stability and longer half-life (Jiménez et al., 2013; Traish et al., 2011) and availability at our institution, but the effects of DHEA on perfusion would also be interesting to measure, since it crosses the blood brain barrier directly. Additionally, we did not measure progesterone levels, so it is not known to what extent progesterone affects cerebral perfusion. Female participants were asked for the date of their last period, but due to the variability in cycle length and ovulation time, it was not possible to confirm the menstrual cycle phase accurately. Future studies examining serum progesterone as well as estradiol, testosterone, and DHEAS levels, with optimal control for menstrual cycle effects would be needed to further corroborate the findings of this study.

2.5.2 Conclusion

This study has replicated the well known sex difference in cerebral perfusion, with women showing significantly higher global perfusion. Moreover, the correlation analyses between perfusion and the steroid hormones revealed a strong modulatory effect of DHEAS on perfusion, with modest sex-dependent correlations with testosterone. These results demonstrate for the first time that steroid hormones contribute to the observed sex difference in perfusion, and that DHEAS in particular may play an important role as an underlying factor accounting for the sex difference in cerebral perfusion.

2.6 Acknowledgements

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2.7 Conflict of interest

The authors declare that they have no conflict of interest.

3 Study B

Age related increase in subcortical GABA/glutamate mediates decrease in short-range subcortical functional connectivity

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3.1 Abstract

Marked changes in brain physiology and structure take place between childhood and adulthood, including changes in functional connectivity and changes in the balance between main excitatory and inhibitory neurotransmitters glutamate (Glu) and γ-Aminobutyric acid (GABA). The balance of these neurotransmitters is thought to underlie neural activity in general and functional connectivity networks in particular, but so far no studies have investigated the relationship of developmental changes in these neurotransmitters and concomitant changes in functional connectivity. GABA+/H_2O and Glu/H_2O levels were acquired in a group of healthy children, adolescents, and adults in a subcortical (basal ganglia) region as well as in a frontal region in adolescents and adults. Our results showed increases with age in GABA+/Glu in both the subcortical and the frontal voxel, which were differentially driven by a significant decrease of Glu/H_2O with age in the subcortical voxel and by a significant increase in GABA+/H_2O with age in the frontal voxel. Using a seed-to-voxel analysis, we were further able to show that functional connectivity decreased with age between the putamen (seed) and other subcortical structures. Age related decrease in subcortical Glu/H_2O mediated the decrease of connectivity in the dorsal putamen specifically. Based on these results, and the potential role of Glu in synaptic pruning, we suggest that age related changes in Glu mediate the decrease of local connectivity during development.

3.2 Introduction

The transition from childhood to adulthood is accompanied by changes in regional grey matter volume, increases in white matter volume (Giedd & Rapoport, 2010), as well as increases in long-range cortico-cortical networks, and decreases in short-range cortical and subcortical networks (Fair, Dosenbach, et al., 2007; Fair et al., 2009; Supekar, Musen, & Menon, 2009). Functional network changes in development are likely to be attributed to putative changes in neurotransmitter levels, such as the major inhibitory and excitatory neurotransmitters γ-Aminobutyric acid (GABA) and glutamate (Glu), respectively. It has been suggested that adjustments of the excitation-inhibition balance on both individual neurons and within networks are accompanied by synaptic elimination during adolescence (Selemon, 2013). The main argument in support of this hypothesis originates from the specificity of loss, i.e. the excitatory synapses are selectively degenerated, while inhibitory synapses are spared in primates (Bourgeois & Rakic, 1993). However, the interaction between developmental changes in GABA and Glu and functional network changes in humans is currently unresolved. GABAergic interneurons not only regulate nearly all key developmental steps in the cortex but also the experience-dependent refinement of local circuits (Di Cristo, 2007), and they play a central role in shaping developing hippocampal networks (Bonifazi et al., 2009). Glu has been linked to synchronizing neuronal networks (Rodriguez et al., 2013). Glu has been reported to be mostly positively associated with functional connectivity (Duncan et al., 2013; Kapogiannis, Reiter, Willette, & Mattson, 2013; Schmaal, Goudriaan, van der Meer, van den Brink, & Veltman, 2012) while GABA has been negatively associated with functional connectivity (Kapogiannis et al., 2013; Stagg et al., 2014).

To our knowledge, only one study investigated GABA and Glu in healthy adolescents (12 – 14 years) in addition to young adults and reported an increase of GABA and a non-significant decrease of Glu with age (Silveri et al., 2013). There are no studies investigating GABA changes in the first years after birth, while a few reports of Glu changes including children and adolescents showed steep increases, reaching a plateau in childhood (Blüml et al., 2013; Degnan et al., 2014).

The aim of this study, therefore, was to answer two questions. First, how do GABA and Glu develop between late childhood to middle adulthood in subcortical and cortical regions? And second, do developmental changes in GABA/Glu contribute to age related changes in neural networks? For the first aim, we acquired GABA+/H_2O and Glu/H_2O levels in healthy children, adolescents, and adults in a subcortical (basal ganglia) region as well as in a cortical (frontal) region in adolescents and adults. We focussed on these two regions since they follow different developmental trajectories, e.g. in grey matter (Østby et al., 2009) and in microstructural maturation (Lebel et al., 2008). For the second aim, we examined the contributions of the subcortical

neurotransmitter levels to the developmental changes of neural networks, assessed by functional connectivity. We expected to find a decrease of Glu/H_2O with age and an increase of GABA+/H_2O with age. We hypothesised that this relative increase in GABA+/Glu with age would be related to changes in local (i.e. short-range) and distal (i.e. long-range) connectivity.

3.3 Material and Methods

3.3.1 Subjects

To investigate developmental changes in the major inhibitory and excitatory neurotransmitter levels, we acquired magnetic resonance spectroscopy (MRS) measures of GABA+/H_2O and Glu/H_2O in healthy children, adolescents, and adults during rest in a subcortical (basal ganglia) and in a frontal voxel. MRS values from the subcortical voxel were obtained from a total of 80 subjects. Of these, 40 were adults (21 females) with a mean age of 32.0 years (*SD*: 9.6, range: 21.0 – 53.3 years) and 40 were children and adolescents (19 females) with a mean age of 13.0 years (*SD*: 3.0, range 8.1 – 18.0 years). The MRS measurement in the frontal voxel was not conducted in one adult subject and only in subjects older than 13 years, resulting in a total of 56 subjects. Of these, 39 were adults (21 females) with a mean age of 32.0 years (*SD*: 9.7, range: 21 – 53.3 years) and 17 adolescents (9 females) with a mean age of 16 years (*SD*: 1.4, range: 13.6 – 18.0 years).

After MRS measurement participants performed a spatial working memory task. Task functional magnetic resonance imaging (fMRI) data was obtained from all children and adolescents and from a subset of adults, resulting in a total of 65 subjects. Of these, 25 were adults (15 females) with a mean age of 32.8 years (*SD*: 9.7, range: 21.0 – 50.6 years). The numbers for children and adolescents remained as stated above for the MRS values. Due to the smaller numbers of subjects for the task fMRI data and MRS measurements in the frontal voxel, we restricted our analysis on the relationship between age related changes in functional connectivity and MRS measurements to the subcortical voxel. No current neurological or psychiatric illness of the subjects was reported by them or their parents, respectively. All subjects gave written informed consent before the experiment. The study was approved by the Ethics Committee of the Canton of Zurich, Switzerland.

3.3.2 MRI-data acquisition

MRI-data was acquired with a 3.0 T GE HD.xt whole-body MRI scanner (GE Healthcare, Milwaukee, WI, USA), using an 8-channel receive-only head coil and a body transmit coil. The MRI protocol included a 3D T1-weighted gradient echo pulse sequence [number of slices = 172, slice thickness (ST) = 1.0 mm, repetition time (TR) = 9.94 ms, echo time (TE) = 2.948 ms, inversion time = 600 ms, field of view (FOV) = 256 mm × 192 mm, flip angle = 8°, matrix = 256 × 192, reconstructed voxel resolution: 1 × 1 × 1 mm] used for positioning of the MRS voxels. GABA-edited MR spectra were acquired using the MEGA-PRESS method (Edden & Barker, 2007) with TE = 68 ms, TR = 2000 ms, 320 averages (160 pairs), and an 8 step phase cycle. We acquired spectra from a 28 x 40 x 25 mm^3 voxel in a left subcortical region centred on the axial slice

where the putamen was the widest, with the anterior border of the voxel aligned with the anterior margin of the putamen (Figure 3-1, top row insets). The frontal voxel was 25 x 40 x 30 mm^3 in size and was positioned as described in Michels et al. (2012). The voxel was centred on the axial slice 1 mm above the superior margin of the left lateral ventricle. The length of the midline was measured on this slice and the centre of the voxel along the anterior-posterior direction was placed at a point 1/3 of the distance down the midline from the anterior margin of the brain and along the left-right direction at a point 1/2 of the distance between the midline and the left lateral border of the brain, perpendicular to the midline (Figure 3-1, bottom row insets). During MRS measurements, subjects were instructed to relax but stay awake. fMRI images were acquired interleaved in 35 axial slices covering the whole brain with a multi-slice echo planar imaging sequence (ST = 3 mm; 0.3 mm skip; 35 slices; TR = 1.925 s; TE = 32 ms; flip angle = 74°; matrix = 64 x 64; FOV = 240 mm).

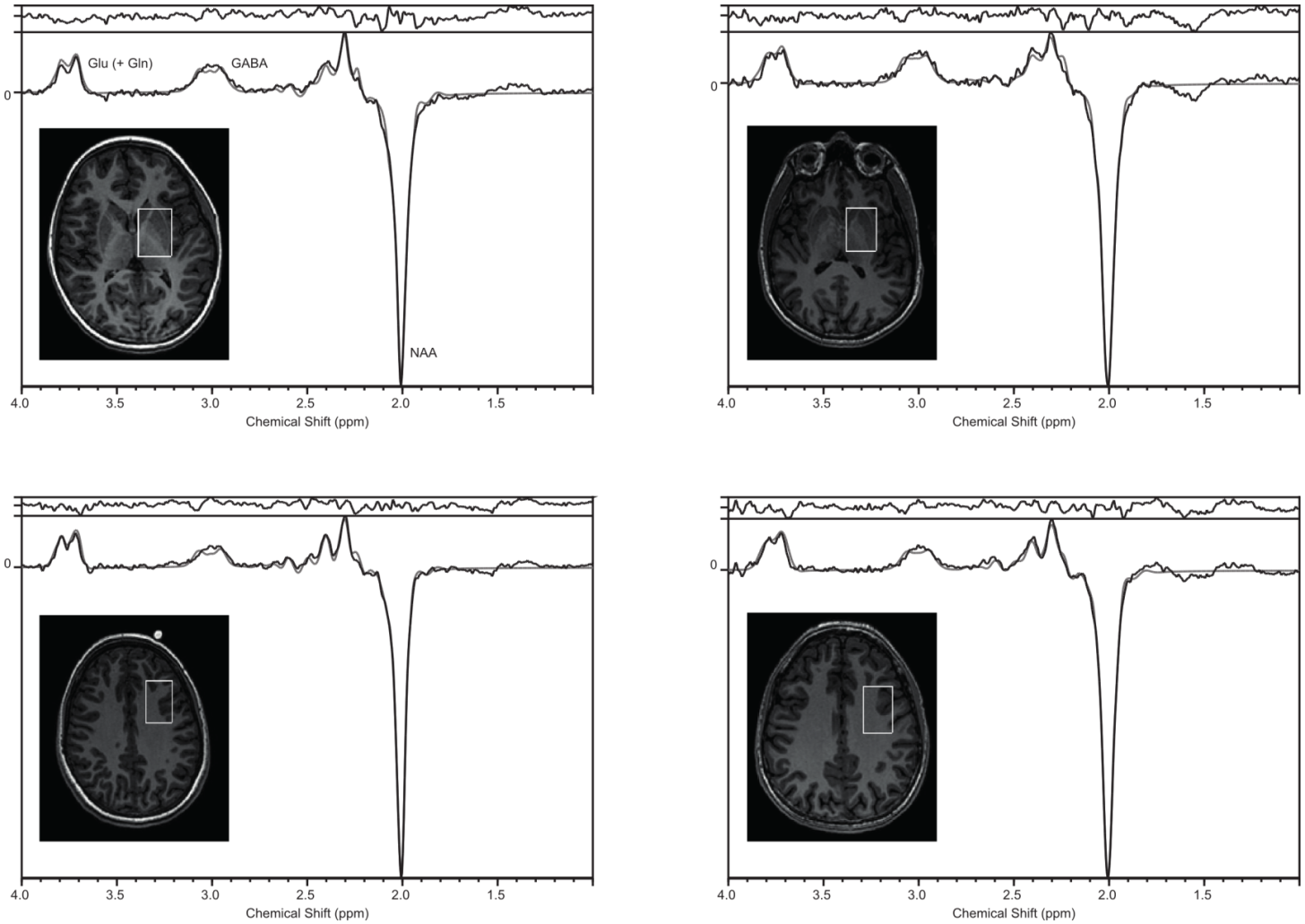

Figure 3-1: Examples of voxel positions and edited spectral fits in the subcortical region (top row) of a child (8.5 years, left) and an adult subject (41.1 years, right), and in the frontal region (bottom row) of an adolescent (14.7 years, left) and an adult subject (41.1 years, right). Spectra are shown in black, with the spectral fits overlaid in grey. The residuals are shown above each spectrum. Glu (+ Gln), glutamate with contributions from glutamine. GABA, γ-Aminobutyric acid. NAA, N-Acetylaspartate.

3.3.3 fMRI task

We investigated functional connectivity related to spontaneous background activity (Fox & Raichle, 2007; Fox, Snyder, Zacks, & Raichle, 2006; Norman-Haignere, McCarthy, Chun, & Turk-Browne, 2012) during a spatial working memory task. The task consisted of a display of eleven circles positioned on a circular grid. Either two or four of the stimulus circles were filled to indicate the positions to be memorised during three different retention intervals (two, four, and six seconds, varying from trial to trial). In the subsequent probe phase of each trial, a question mark was shown in one of the eleven circles and subjects indicated by a button press (LuminaBox) whether or not that circle had been filled. The task lasted 14 minutes and contained 72 trials in total, with half of the trials showing two positions to memorise and half with four (at most two trials with the same number in succession). Null trials were included (fixation cross for two, four, and six seconds) after four trials and motivation trials after twelve trials, displaying the number of correctly answered trials and a smile. The task was implemented in Presentation version 16 (Neurobehavioral Systems, Inc.) and synchronized to the fMRI acquisition. During the MRI acquisition, the task was projected onto a screen at the foot of the MR table, which subjects viewed via a mirror.

3.3.4 MRS analysis

T1-weighted images were segmented in native space using SPM8 with the "New Segment" module (Wellcome Department of Cognitive Neurology, London, UK) running in MATLAB 2012b (The MathWorks, Inc., Natick, Massachusetts, USA) to correct the MRS results for partial volume cerebrospinal fluid contamination and different water compartment relaxation times (Gasparovic et al., 2006). The spectra were coil combined with weighting factors derived from the first point of the free induction decay signal from the unsuppressed water lines acquired with each coil. Water scaled metabolite concentrations were derived with LCModel version 6.3-1B (Provencher, 1993). The edited spectra were analyzed with a simulated basis set including basis spectra for GABA, glutathione, N-Acetylaspartate (NAA), glutamine, Glu, and N-Acetylaspartylglutamate (Figure 3-1). GABA findings described subsequently are reported as GABA+ due to the contribution of a macromolecular component to the edited peak at 3 ppm in the assessment of GABA with MEGA-PRESS. GABA+/H_2O and Glu/H_2O were quantified from the edited spectra, using the control parameter sptype='mega-press-2'. The Cramer Rao Lower Bounds of the spectral fit for all GABA+/H_2O and Glu/H_2O values were below 20% for all participants.

3.3.5 Statistical analyses

Correlations (Spearman's r_s) between age and MRS values (GABA+/Glu, GABA+/H_2O, Glu/H_2O) are reported. Statistics and plots were performed using R (version 3.1.0, rcorr function from the Hmisc package and ggplot from the ggplot2 package).

3.3.6 Functional connectivity analyses

Functional connectivity was assessed with the conn toolbox version 14.c (Whitfield-Gabrieli & Nieto-Castanon, 2012) using standard fMRI preprocessing. Spatial preprocessing of the task fMRI data included slice-timing correction, realignment, coregistration of functional volumes to the anatomical volumes, and smoothing with a Gaussian filter of 8 mm. The voxel size for analyses was 2 mm. T1-weighted anatomical volumes were segmented into grey matter, white matter, and cerebrospinal fluid (CSF) and the resulting transformation parameters were used to normalise the anatomical and functional images. These preprocessed images were further band-pass filtered (0.01 – 0.1 Hz), and non-neuronal contributions from white matter and CSF were regressed out (5 principle components) at the subject level using the aCompCor strategy (Behzadi, Restom, Liau, & Liu, 2007) implemented in the conn toolbox. Additionally, each subject's realignment parameters (three translation, three rotation parameters, and their first temporal derivates) were included as confounding factors. Finally, event-related task effects were also regressed out with five regressors (and their first temporal derivatives) consisting of two regressors modelling the retention phase with different durations of correctly answered trials (starting from stimulus onset and lasting until probe onset) for the two- and the four-circle-trials respectively, two regressors modelling the retrieval phase of correctly answered trials (starting from probe onset and lasting until button press) for the two- and the four-circle-trials respectively, and one regressor modelling all button presses (duration of 0 s). This approach of analysing functional connectivity from residual time courses of event-related task fMRI data has been described by Fair et al. (2007) and applied by several studies (e.g. J. J. Harris, Reynell, & Attwell, 2011; Zhang, Hu, Bednarski, Erdman, & Li, 2014). These spontaneous correlations are meaningful and may help characterize the systems level interactions subserving cognitive processes (Norman-Haignere et al., 2012). Since the MRS subcortical voxel was centrally placed on the left putamen, this region was chosen as the seed region for the connectivity analyses. The region of interest was taken from the Harvard-Oxford Subcortical Structural Atlas available in FSL (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/). The average blood-oxygen-level dependent (BOLD) time series was computed across all the voxels within the left putamen and used for the seed-to-voxel (whole brain) bivariate correlation analyses. For second level analyses, i.e. regression models with age and the MRS values as between-subject effects, a Fisher transformation (inverse hyperbolic tangent function) was applied to the correlation measures in order to improve the normal-

ity assumptions of standard second-level general linear models (Whitfield-Gabrieli & Nieto-Castanon, 2012). Separate regression models were run in the conn toolbox across all available subjects to evaluate the association between seed-to-voxel connectivity (left putamen as seed) and the variables age and the different MRS values (GABA+/Glu, GABA+/H_2O, Glu/H_2O from the subcortical voxel) as between-subject effects. Results reported survived a height threshold of $p < .001$ (uncorrected) and an extent threshold of family wise error-corrected $p < .05$ at the cluster level.

As mentioned in the subject section, group sizes for different analyses varied due to differences in available data per subject. A summary of the numbers for the respective analyses is provided here: correlation between age and MRS values from the subcortical voxel: age range = 8.1 – 53.3 years, $n = 80$, 40 females; correlation between age and MRS values from the frontal voxel: age range = 13.6 – 53.3 years, $n = 56$, 30 females; regressions of functional connectivity by age and functional connectivity by MRS values from the subcortical voxel: age range = 8.1 – 50.6 years, $n = 65$, 34 females.

3.4 Results

3.4.1 Correlations MRS values with age

In the subcortical voxel (Figure 3-2, top row), GABA+/Glu showed a positive correlation with age (r_s = .58, p < .001, left). A negative correlation between Glu/H_2O and age was observed (r_s = -.61, p < .001, right) while the correlation between GABA+/H_2O and age was not significant (r_s = -.07, p = .55, middle).

In the frontal voxel (Figure 3-2, bottom row), GABA+/Glu also showed a positive correlation with age (r_s = .42, p = .001, left). In contrast to the subcortical voxel, a positive correlation between GABA+/H_2O and age was observed (r_s = .30, p = .02, middle), while the correlation between Glu/H_2O and age was not significant (r_s = -.18, p = .18, right).

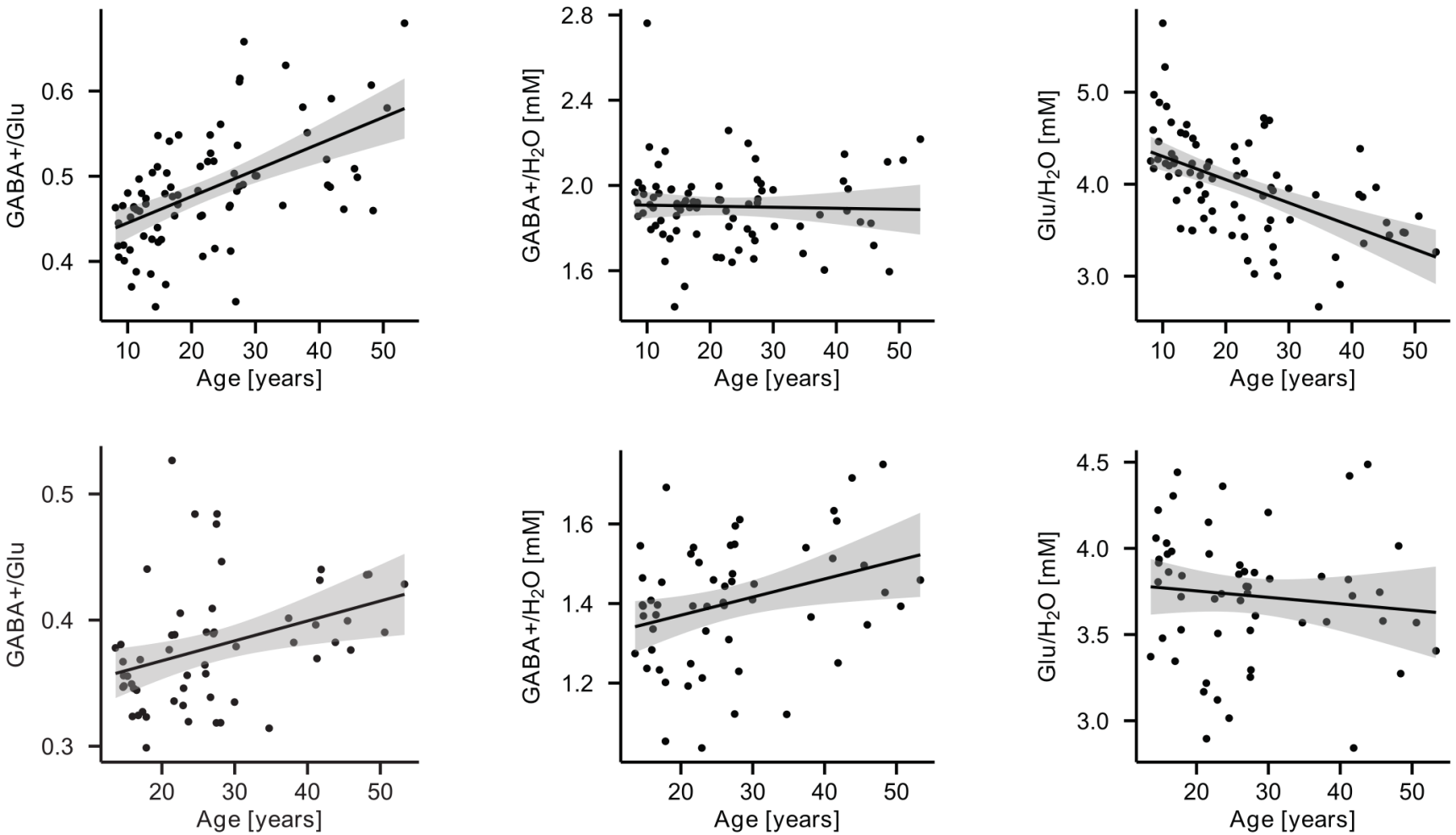

Figure 3-2: Spectroscopic measures in the subcortical voxel (top row) and the frontal voxel (bottom row) are differentially correlated with age. GABA+/Glu (left column) showed a significant increase with age in both regions (r_s = .58, p < .001 for the subcortical region and r_s = .42, p = .001 for the frontal region). GABA+/H_2O (middle column) showed a significant increase with age in the frontal region (r_s = .30, p = .02; not significant in the subcortical region, r_s = -.07, p = .55). Glu/H_2O (right column) showed a significant decrease with age in the subcortical region (r_s = -.61, p < .001; not significant in the frontal region, r_s = -.18, p = .18).

3.4.2 Correlations functional connectivity with age and MRS values

Figure 3-3 and Table 3-1 illustrate the results of the correlation analyses of functional connectivity with age and MRS values.

3.4.2.1 Age

The functional connectivity showed a negative correlation with age in a cluster encompassing the left putamen and neighbouring regions, i.e. left pallidum, thalamus, amygdala, hippocampus, and parahippocampal gyrus (Figure 3-3, first row).

3.4.2.2 MRS values from the subcortical voxel

GABA+/Glu values correlated negatively with functional connectivity in a cluster encompassing the left dorsal putamen and adjacent external capsule (Figure 3-3, second row). Regarding Glu/H_2O and $GABA+/H_2O$ individually, a negative correlation between Glu/H_2O and functional connectivity was observed in a cluster encompassing the left ventral putamen and orbitofrontal cortex (Figure 3-3, third row in blue). This cluster did not overlap with the above mentioned cluster correlating negatively with age. A positive correlation between Glu/H_2O and functional connectivity was observed in a cluster encompassing the left dorsal putamen and adjacent external capsule (Figure 3-3, third row in red). This cluster largely overlapped with the above mentioned cluster correlating negatively with age (Figure 3-3, fourth row in yellow). Several other regions that correlated with age, e.g. thalamus and pallidum, showed no significant correlation with Glu. No significant correlation between $GABA+/H_2O$ and functional connectivity was observed. Since the co-localization between age related changes in connectivity and Glu/H_2O related changes in connectivity suggested a Glu/H_2O mediated change in connectivity with age, we tested the correlation between age and putamen connectivity after controlling for Glu/H_2O. The results showed that the correlation between age and connectivity remained significant in all regions except for the dorsal putamen, indicating that the connectivity with the dorsal putamen was mediated by age related changes in Glu/H_2O. Similar results were found when we controlled for the GABA+/Glu ratio.

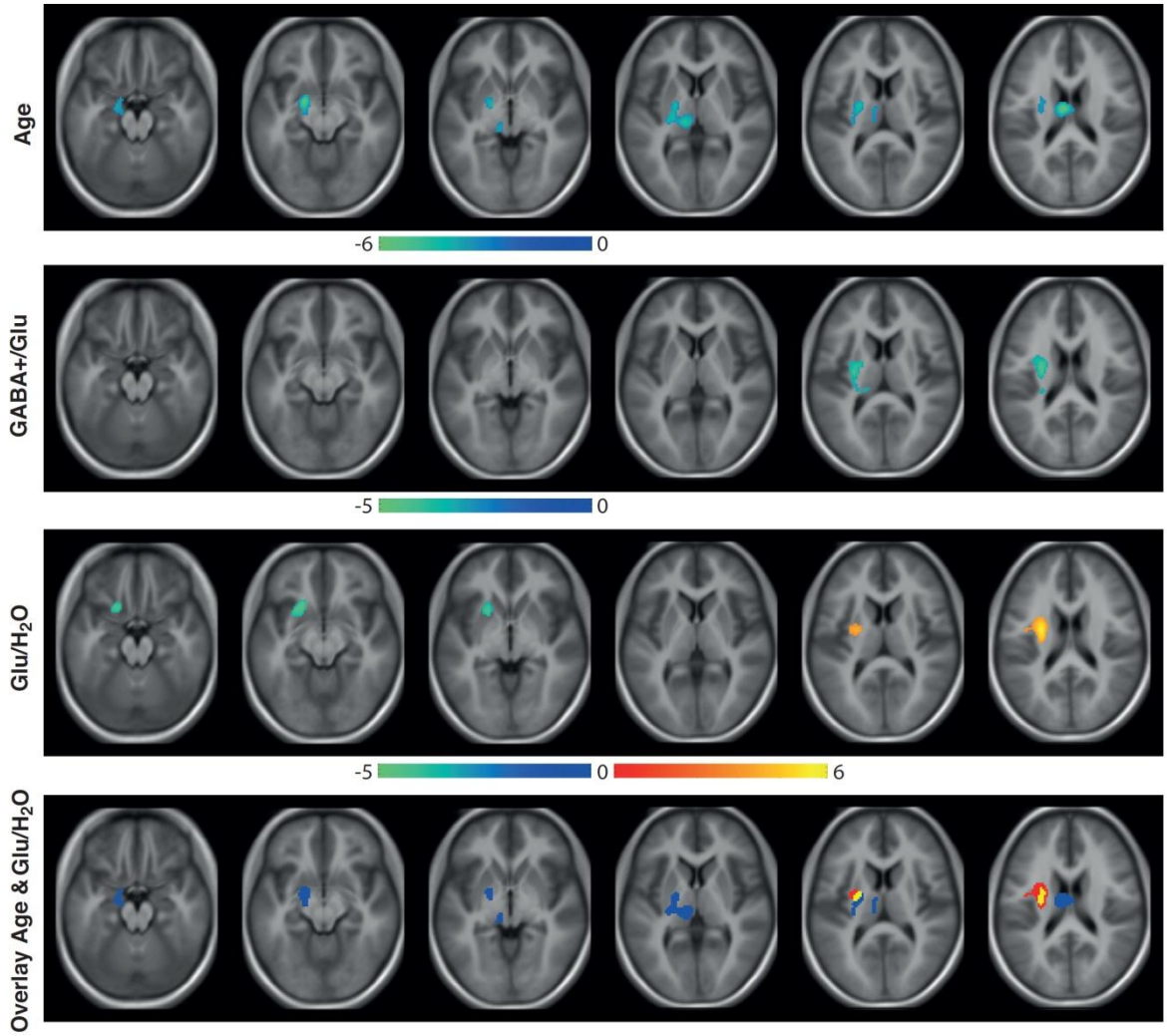

Figure 3-3: Correlations of functional connectivity (left putamen as seed region) with age, and the spectroscopic measures from the subcortical region. First row, functional connectivity correlated negatively with age in a cluster encompassing the left putamen and neighbouring regions, i.e. left pallidum, thalamus, amygdala, hippocampus, and parahippocampal gyrus. Second row, functional connectivity correlated negatively with GABA+/Glu values in a cluster encompassing the left dorsal putamen and adjacent external capsule. Third row, functional connectivity correlated negatively with Glu/H_2O in a cluster encompassing the left ventral putamen and orbitofrontal cortex and positively in a cluster encompassing the left dorsal putamen and adjacent external capsule. Fourth row, overlay of the correlations between functional connectivity and age (blue), Glu/H_2O (red), and their overlap (yellow). The cluster showing a correlation with age overlapped in the dorsal putamen with the Glu/H_2O correlation cluster but not in other regions such as thalamus or pallidum. Results reported survived a height threshold of $p < .001$ (uncorrected) and an extent threshold of family wise error-corrected $p < .05$ at the cluster level. Colour bar shows T values. Slices are at MNI z-coordinates [-20 -12 -4 4 12 20].

Table 3-1: Correlations of functional connectivity (left putamen as seed region) with age, and the spectroscopic measures from the subcortical region

Correlation	Region	k	x y z (mm)			*T*	*p*
Age	Left thalamus	1403	-6	-12	20	-6.23	< .001
	Left pallidum / amygdala / hippocampus		-18	-4	-10	-5.9	
	Left putamen / pallidum		-22	-8	8	-5.34	
GABA+/Glu	Left dorsal putamen and external capsule	487	-28	-14	16	-5.26	.009
Glu/H_2O	Left dorsal putamen and external capsule	386	-28	-6	18	5.85	.024
Glu/H_2O	Left ventral putamen / frontal orbital cortex	330	-20	18	-16	-4.71	.042

Results reported survived a height threshold of $p < .001$ (uncorrected) and an extent threshold of family wise error-corrected $p < .05$ at the cluster level. k, cluster size. x y z (mm), peak MNI coordinates.

3.5 Discussion

Our hypothesis of an age related increase in the GABA+/Glu ratio was confirmed in both regions investigated. Interestingly, the individual relationships for GABA+/H_2O and Glu/H_2O with age were different in the two regions measured yielding a significant negative correlation with Glu/H_2O in the subcortical voxel and positive correlation with GABA+/H_2O in the frontal voxel, respectively. The age-related changes in Glu/H_2O observed in the present study are consistent with reports of a negative correlation between Glu and age in the basal ganglia (Chang et al., 2009) and of a lack of correlation between Glu and age in the frontal cortex (Chang et al., 2009; Sailasuta, Ernst, & Chang, 2008). The positive correlation between GABA+/H_2O and age in the frontal cortex, but not in other regions, is in line with previous studies in adolescents and adults (Gaetz et al., 2014; Silveri et al., 2013). An explanation for these results might be that the developmental trajectories of Glu and GABA are region specific and may also support varying functions in different regions. One could speculate that Glu supports pruning of subcortical grey matter and concurrent rewiring of grey matter structures (Lebel et al., 2008), while GABA supports myelination of frontal white matter trajectories by controlling oligodendrocyte precursor cell activity (Vélez-Fort, Audinat, & Angulo, 2012). Further studies would be necessary to associate structural changes in grey and white matter with Glu and GABA levels across the full developmental trajectory. Taken together, this is the first study reporting that the developmental change in this ratio depends on both the region and neurotransmitter investigated.

A negative correlation was observed between GABA+/Glu from the subcortical voxel and local functional connectivity in the dorsal putamen. In this region we also observed a negative correlation between age and local functional connectivity. This decrease of functional connectivity with age was also observed in other neighbouring regions such as pallidum, thalamus and hippocampus, i.e. regions that showed no correlation with GABA+/Glu. We propose that Glu/H_2O mediates the developmental decrease of local functional connectivity in the dorsal putamen. In support of this notion, we found a positive correlation between Glu/H_2O and functional connectivity in this region, while the correlation between functional connectivity and age disappeared after controlling for Glu/H_2O levels. Glu has previously been shown to be positively associated with functional connectivity (Duncan et al., 2013; Kapogiannis et al., 2013; Schmaal et al., 2012) as well as negatively (Duncan et al., 2013), depending on the target region of interest. Our finding of both positive and negative correlations between Glu/H_2O and functional connectivity may suggest a dissociation of the effect of Glu/H_2O, since only the positive association was also related to developmental changes in functional connectivity. We did not find any significant correlation between connectivity and GABA+/H_2O analysed separately. Other studies reported a negative association between GABA+/H_2O and functional connectivity (Kapogiannis et al., 2013; Stagg et al., 2014). Both these studies included adult subjects only and both used other metabo-

lites (i.e. creatine and NAA) for quantification of the GABA values, whereas water scaled concentrations were used in the present study. It is important to note that both NAA and creatine have been shown to change with age in adults (Duncan et al., 2014; Haga et al., 2009) and in younger (< 18 years) subjects (Blüml et al., 2013). For developmental MRS studies, the unsuppressed water signal may therefore represent a more stable calibration standard for metabolite quantification.

Neuronal activity appears to be primarily affected by the excitation-inhibition balance, and variations in this balance are thought to underlie the BOLD signal as an indirect measure of neuronal activity (Isaacson & Scanziani, 2011; Lauritzen et al., 2012; Logothetis, 2008). Changes from childhood to adulthood in the spontaneous coherence of the BOLD signal may therefore be related to developmental changes in the excitation-inhibition balance. Developmental decreases of local functional connectivity have been reported before (Fair, Dosenbach, et al., 2007; Fair et al., 2009; Supekar et al., 2009) and increases in synaptic pruning and myelination have been suggested to underlie these findings (Power et al., 2010). Glu receptor-mediated synaptic plasticity appears to be an important factor for synaptic pruning in brain development, which probably involves the adjustment of the excitation-inhibition balance on both individual neurons and within networks (Selemon, 2013). This adjustment includes a relative gain in the inhibitory/excitatory synapse ratio, which is presumably mirrored in our finding of an increase of the GABA+/Glu ratio with age. Our observed developmental decrease of Glu/H_2O may reflect an increase in synaptic pruning, which in turn leads to a decrease in functional connectivity. We thus propose that a decrease in Glu/H_2O from childhood to adulthood can lead to a decrease of local (i.e. short-range) connectivity via an associated increase in synaptic pruning. The imbalance in the excitation-inhibition ratio has been linked to neurodevelopmental disorders (Gatto & Broadie, 2010) and our work thus is significant in further elucidating the role of Glu in fine tuning functional connectivity networks.

It is important to note that both GABA and Glu are present in the brain in different pools (e.g. intra- vs. extracellular levels; Rae, 2013), which cannot be disentangled using current MRS measures. Nevertheless, the concentrations in these pools appear to be balanced and their total concentrations (as measured in the present study) are neurophysiologically relevant (Kapogiannis et al., 2013). In addition, it is likely that for both GABA and Glu any pool may be drawn on as a source for neurotransmitter GABA and Glu (Rae, 2013). Further, it is currently not possible to account for the macromolecular contributions to the GABA signal to a satisfactory degree without other detrimental effects on data quality or acquisition time (Mullins et al., 2014). Since the macromolecular contribution might be associated with age (Aufhaus et al., 2013), further development of techniques accounting for this putatively confounding factor are especially relevant for future studies investigating developmental changes in GABA. Motion artefacts have been

discussed to bias estimates of functional connectivity, which might be of particular importance for studies investigating developmental changes because motion can be related to subject age (Satterthwaite, Wolf, et al., 2013). Yet, the effects of in-scanner motion are likely to exert minimal effects on the present finding of an age-related decrease in local (short-range) connectivity. First, because the impact of motion appears to be less strong in age-negative connections than in age-positive connections (Chai, Ofen, Gabrieli, & Whitfield-Gabrieli, 2013; Satterthwaite, Wolf, et al., 2013), second because subcortical areas are amongst the least affected by head motion since the pivot of the head rotation lies near to these regions (Satterthwaite, Elliott, et al., 2013). We used task related background activity to investigate developmental changes in functional connectivity. Previous studies have shown that "hard-wired", i.e. anatomical, connections underlie functional connectivity networks (Damoiseaux & Greicius, 2009), and that task instruction (Norman-Haignere et al., 2012) and hormonal concentrations (e.g. oxytocin; Kirsch et al., 2005) can modulate connectivity. Although the role of Glu across different tasks is unknown, several studies have reported the positive correlation of Glu also with resting state functional connectivity (Duncan et al., 2013; Kapogiannis et al., 2013; Schmaal et al., 2012). Our results thus extend the knowledge by demonstrating this positive correlation also in task-related background activity and we argue that the positive relationship between Glu and functional connectivity is not limited to resting state data, but may represent a more general role of Glu in the modulation of functional connectivity. Finally, the role of the putamen in the context of working memory has mainly been attributed to filtering efficiency, i.e. hindering irrelevant information from entering working memory (Baier et al., 2010; Frank, Loughry, & O'Reilly, 2001; McNab & Klingberg, 2008). The ability to inhibit irrelevant information increases with age (Robert, Borella, Fagot, Lecerf, & de Ribaupierre, 2009), suggesting a potential role of striatal regions in the development of working memory networks possibly in cooperation with fronto-parietal and hippocampal networks (Finn, Sheridan, Kam, Hinshaw, & D'Esposito, 2010; von Allmen, Wurmitzer, & Klaver, 2014). Our results of a decrease in functional connectivity in striatal regions might reflect the fine-tuning of these filtering processes, possibly mediated by a concomitant developmental decrease of Glu/H_2O mirroring increased synaptic pruning. Future studies also investigating other tasks, especially with the addition of irrelevant stimuli, are needed to further elucidate this proposed role of Glu.

In conclusion, the present results show increases with age in GABA+/Glu in both a subcortical (basal ganglia) and a frontal voxel, which are differentially driven by a decrease of Glu with age in the subcortical voxel and by an increase in GABA+ with age in the frontal voxel. We further found an age related decrease in connectivity between the putamen and other subcortical structures. The age related decrease in connectivity in the dorsal putamen was mediated by subcorti-

cal Glu. Based on these results and the potential role of Glu in synaptic pruning, we suggest that age related changes in Glu mediate the decrease of local connectivity during development.

3.6 Conflict of Interest

The authors declare no competing financial interests

3.7 Acknowledgements

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4 General Discussion

The general aim of this thesis was to examine the relationships between major system markers for typical human brain development. In order to achieve this, multimodal MR imaging techniques, MR spectroscopy, and hormone assays were used to acquire measures of a selected set of major system markers including cerebral perfusion, functional connectivity, neurotransmitters, and steroid hormones. While much research has already been conducted on these measures separately, less is known about the relationships between them. Moreover, there is a lack of studies linking the developmental courses of these measures. Thus, we investigated the relationships between selected system markers in Studies A and B. These studies provided us with further insight into the associations between the markers and they raised new questions about the possible mechanisms governing the interplay of different systems. Subsequently, the findings of these studies will be summarised and discussed, which includes a critical view of the methodology used and a presentation of thoughts on possible future directions.

4.1 Steroid hormones and sex differences in the brain – could DHEAS be a major factor?

Sex differences in brain structure and function as well as in the prevalence and onset of many psychiatric diseases are well known (e. g. Ruigrok et al., 2014). Since many of these differences only emerge in adolescence (i.e. with puberty; Lenroot & Giedd, 2010; Paus et al., 2008) and some are attenuated in older age (i.e. after menopause; Gur & Gur, 1990), it seems intuitive to consider the variations in the (sex) steroid hormone levels between men and women as possible underlying factors for the sex differences observed. However, regarding the well known sex difference in cerebral perfusion (e.g. Devous et al., 1986), no study so far has investigated the association between steroid hormone levels and the fact that women consistently show higher perfusion than men. In Study A we thus conducted the first investigation of the relationship between several steroid hormone levels and cerebral perfusion measured with ASL. Besides replicating the known finding that women show higher regional and global perfusion, we were able to demonstrate that there are indeed significant relationships between steroid hormone levels and cerebral perfusion. Surprisingly, we did not find a significant correlation between oestradiol and perfusion, nor a strong correlation between testosterone and perfusion despite the known modulatory effects these hormones have on a variety of vascular properties (Krause et al., 2006). Interestingly, DHEAS showed a very strong negative correlation with both regional and global cerebral perfusion. Our finding of a negative association suggests a role of DHEAS in the sex difference in cerebral perfusion: If DHEAS is indeed a factor contributing to the sex difference in perfusion, a negative association between DHEAS levels and perfusion is expected since a) perfusion is higher in women (e.g. Devous et al., 1986) but b) DHEAS levels are higher in men (e.g. Šulcová et al., 1997).

4.1.1 Possible mechanisms of DHEAS affecting perfusion

There are several possible mechanisms by which DHEAS could affect perfusion. In addition to its synthesis in the human adrenal glands, DHEA(S) is also synthesised de novo in the human brain (Baulieu & Robel, 1998) and has subsequently been termed "neurosteroid". DHEAS is one of the most important neurosteroids and the broad effects of DHEAS in the brain have been well documented (e.g. in Dong & Zheng, 2012). These effects include the modulation of the synaptic release of acetylcholine, dopamine, GABA, and glutamate by DHEAS, as well as the role of DHEAS as a modulator of $GABA_A$ and glutamate receptors. For example, our finding of a negative relationship between DHEAS and perfusion could be explained by combining the knowledge that DHEAS is a $GABA_A$ receptor antagonist and the reports of a positive association between GABA and cerebral blood flow (Donahue, Near, Blicher, & Jezzard, 2010). Another possible route of action of DHEA(S) on brain physiology lies in the fact that DHEA(S) is a precursor to the sex

steroids testosterone and oestradiol and thus our results could also, in part, be interpreted as a consequence of the local conversion of DHEAS into the sex steroids (Fokidis et al., in press). The amount of circulating DHEAS for instance may determine the rate of conversion into testosterone and/or oestradiol in other tissues, including the brain. In fact, these multiple ways of action and intricate interplay of the different hormones might be one of the reasons why we did not observe a strong association between perfusion and either testosterone or oestradiol. However, much remains unknown on this topic, especially since these mechanisms most likely differ again between men and women. For example, Lenroot & Giedd (2010) discussed that while the modulation of local oestrogen by the rapid conversion of testosterone to oestradiol permits the effectiveness of oestrogen as a neurotransmitter, it is not known how the much lower endogenous concentrations of testosterone in females might affect this pathway.

4.1.2 Methodological considerations and possible future approaches

Besides these considerations rather on the theoretical side, it is also important to mention some methodological considerations regarding Study A. Investigations including hormone measurements entail their own set of challenges, as already mentioned in Chapter 1.5. Diurnal variations are inherent to cortisol levels and while we did collect these samples within a reasonable time window for all subjects, this measure proved to be too limited to draw firm conclusions from the analyses including cortisol levels. Future studies might consider increasing experimental control by additionally taking baseline measurements of cortisol or by the collection of several samples over the measurement session which would allow the use of area under the curve (AUC) measures as a more reliable marker for the average level of cortisol (Adam & Kumari, 2009). Other steps recommended for experimental control include the instruction of study participants to avoid food intake as well as exercise for at least two hours before sampling (Hansen, Garde, & Persson, 2008).

There are also manifold methodological challenges accompanying investigations of sex steroid effects. On the part of the participants, some variation is introduced by their taking medications affecting these hormones (e.g. hormonal contraceptives) and/or, in the case of women, by fluctuating hormone levels due to the menstrual cycle. To include only women not taking hormonal contraceptives poses a challenge for recruiting, especially for groups of younger women, although it is feasible with additional effort as done in this study. The potential confounding factor of female participants being in different menstrual cycle phases could be met by several ways. One could obtain additional hormone values (e.g. luteinizing hormone) to check for menstrual cycle phase as done in other studies (e.g. Witte et al., 2010) or one could invite women only at specific times of their cycle. However, the experience from our study was that many participating women reported astonishing variability in the length of their cycles, particu-

larly if they are not under hormonal contraceptives. This stunning inter- and even intra-individual variability in cycle length and timing of cycle phases (including ovulation time) is well-established even in normal, healthy women (Fehring, Schneider, & Raviele, 2006; Geirsson, 1991; Yan Liu, Gold, Lasley, & Johnson, 2004). Thus, measuring women only at specific times in their cycles, e.g. shortly after their menstruation, requires a large amount of flexibility not only on the part of the investigators and participants but also on the part of the availability of MRI facilities, which can be problematic especially if the MRI facilities are primarily used for clinical purposes within a hospital as was the case in our study. While this elaborate controlling was thus not feasible in our study, we recorded the onset of the last period in the female subjects to estimate their current cycle phase. However, the aforementioned large variability in cycle length made it unfeasible to estimate the women's current cycle phase to a satisfactory degree, even when additionally taking into account their respective oestradiol levels since these levels can also vary widely between different women, as well as between cycles for the same women (Stricker et al., 2006). Nevertheless, future studies with the primary aim to investigate menstrual cycle effects should of course invest the extra effort to determine the cycle phase in a sure manner and the additional measurement of luteinizing hormone and possibly progesterone seems to be the most efficient way.

Finally, while acquiring perfusion ASL images in children is relatively simple as demonstrated in this and other studies (e.g. Taki et al., 2011a), obtaining hormone levels in young children proved to be much harder. The reliable measurement of hormone concentrations from serum samples is hampered by the lower limits of detection (sensitivity) of the currently widely available hormone assays. These lower limits of detection are quite often above the actual concentrations of the hormones in the circulation in young (prepubertal) children (Rahhal et al., 2008). This issue also affected our study and it was not possible to obtain sex steroid levels in most of the participating children. Liquid chromatography/tandem mass spectrometry (LC/MSMS) has been shown to provide higher sensitivity and accuracy of measurements of hormone values (Rahhal et al., 2008), and Rahhal et al. (2008) predicted that these analytical tools will become the preferred method for evaluation of oestradiol and testosterone, once they are more widely available.

Once this technical hurdle has been overcome, studies investigating developmental changes in the sex steroid concentrations and their relation to concomitant changes in brain perfusion (and other cerebral markers) are of high interest since a body of evidence points towards puberty and its associated hormonal changes to play an important role in the emergence of cerebral sex differences and in the onset of many psychiatric disorders (cf. Chapter 1.2). This might not only apply to the sex steroid hormones, but also to DHEAS. For instance, there is evidence that DHEA levels in adolescents are associated with subsequent major depression (Goodyer,

Herbert, Tamplin, & Altham, 2000). Further, virtually all brain regions that were found to show both the sex difference in perfusion and the correlation between DHEAS and perfusion in our study also show high density of oestrogen and androgen receptors in homologous regions in animals during early development (Goldstein et al., 2001) and there is some evidence for developmental changes, especially during puberty, in sex steroid receptor expression (Sugiyama et al., 2008). Thus, using more sensitive hormone assays capable of quantifying the very low steroid levels in prepubertal children, future studies that investigate the role of developmental changes in steroid hormone levels and the accompanying changes in cerebral perfusion (and other brain markers) are needed. These investigations might lend more insight into the role of different brain markers as underlying factors for the striking differences in the prevalence for men and women of many psychiatric disorders as well as for their emergence during puberty.

4.2 Establishing a link between the developmental changes in neurotransmitters and in functional connectivity

Apart from marked structural changes from childhood to adulthood (e.g. Lebel et al., 2008; Lenroot et al., 2007), functional connectivity changes with development are also well-documented and are characterised by progressive segregation and integration and a concomitant decline in short-range (i.e. local) connectivity strength and rise in long-range (i.e. distal) connectivity strength (e.g. Tau & Peterson, 2009). Developmental changes in the major excitatory and inhibitory neurotransmitters (GABA and glutamate, respectively) are possibly underlying these functional connectivity changes since the balance of these neurotransmitters is thought to constitute neural activity in general, and functional connectivity in particular (Duncan et al., 2014). However, reports on developmental changes in GABA and glutamate levels are scarce, and so far no study has reported findings on the relationship between these levels and developmental changes in functional connectivity. Therefore, our Study B had two aims: first, to examine the developmental changes in water scaled GABA and glutamate levels (in the following abbreviated as GABA+/H_2O and glutamate/H_2O, respectively), and second, to investigate their relationship with accompanying changes in functional connectivity. Our results showed an age-related increase in the GABA+/glutamate ratio in both regions measured. Interestingly, this increase was differentially driven by a rise of GABA+/H_2O in the frontal region and by a decline of glutamate/H_2O in the subcortical voxel. Moreover, we found an age-related decrease of local functional connectivity with the putamen as the seed region and this decrease was mediated by subcortical glutamate/H_2O in the dorsal putamen but not in other neighbouring regions.

4.2.1 Age related changes in GABA+/H_2O and glutamate/H_2O vary between regions

Our findings regarding the age-related changes in GABA+/H_2O and glutamate/H_2O are largely consistent with previous findings in adults (e.g. Chang et al., 2009; Sailasuta et al., 2008), as well as the few previous studies including adolescents and adults (e.g. Gaetz et al., 2014; Silveri et al., 2013). What is striking in our and other reports is the fact that the association between age and the neurotransmitter levels measured seem to vary to a large degree between different regions. This is not only true for investigations of age-related changes, but also for investigations of pathological changes (e.g. in GABA; Puts & Edden, 2012). Converging evidence thus suggests that neurotransmitter levels are not necessarily the same across different regions, let alone the whole brain. Future studies examining these levels in multiple regions are therefore warranted. The so-called chemical shift imaging (CSI; also referred to magnetic resonance spectroscopic imaging, MRSI) might prove to be a useful technique since it allows the collection of information from multiple voxels in the same acquisition (Boer & Klomp, 2014). However, the acquisition

times of both single voxel MRS measurements and MRSI are quite long, which may be a limiting factor especially for developmental studies since long scanning sessions are less well tolerated by young subjects. Several acceleration approaches exist and the development of new techniques is ongoing, it is thus hoped that future studies might be able to examine the heterogeneity of the different metabolites in adults as well as in children and adolescents (Boer & Klomp, 2014).

4.2.2 GABA+/H_2O and glutamate/H_2O are differentially associated with developmental changes in functional connectivity

Our results on the relationship between developmental changes in the neurotransmitters and functional connectivity fit broadly with the existing literature in adults. That is, the positive association between glutamate and functional connectivity has previously been demonstrated in several studies, and some studies reported a negative relationship between GABA and functional connectivity (for a review, cf. Duncan et al., 2014). We did not observe a significant relationship between GABA+/H_2O and functional connectivity in our sample, which might be due to different choices made in Study B compared to previous studies concerning the reference or scaling for the neurotransmitter quantification. Specifically, we decided to use the unsuppressed water signal as the reference since the previously used metabolites (NAA and creatine) have been shown to change with age in adults (Duncan et al., 2014; Haga et al., 2009) and in children and adolescents (Blüml et al., 2013). Furthermore, these metabolites used for scaling in other studies might also differ between regions. We therefore expected the unsuppressed water signal to be a more stable calibration standard for metabolite quantification for our developmental study. Future studies comparing the different scaling methods in a developmental subject group might be necessary to be better able to weigh their respective advantages and disadvantages against each other (for a general discussion, cf. Mullins et al., 2014; for arguments concerning developmental studies, cf. Silveri et al., 2013).

4.2.3 Glutamate potentially mediates the decrease of local connectivity with age

These current technical shortcomings notwithstanding, our findings especially regarding the potential role of glutamate in mediating developmental decreases in local functional connectivity are intriguing since evidence showed that glutamate could be necessary for synchronising neural networks (Rodriguez et al., 2013). Our finding of a developmental decrease of glutamate coupled with the one of a positive association between glutamate and functional connectivity could therefore indicate a major role of glutamate in the fine-tuning of neural circuits from childhood to adulthood. This line of thought is supported by our observation that the decrease of local functional connectivity disappeared in the dorsal putamen when we controlled for the glu-

tamate/H_2O levels while this did not affect other regions that showed a decrease with age but no correlation with glutamate/H_2O. In other words, in regions where glutamate is associated with functional connectivity, the developmental decrease in glutamate seen in our and other studies possibly leads to the often demonstrated decrease in local functional connectivity from childhood to adulthood.

How could glutamate mediate this developmental effect? Glutamate binds to several types of ionotropic and metabotropic receptor types (for further details, cf. Rae, 2013) and these various receptors have differential neural effects and activity timings (Duncan et al., 2014). For example, glutamate can act both synaptically and extrasynaptically and its effects differ in each location. Neuronal glutamate exerts the classical excitatory effect at the synapse, while extrasynaptic glutamate (via diffusion from the synapse or release from astrocytes) can have multiple effects on various systems including the modulation of blood flow and, as mentioned above, the regulation of neuronal synchronisation (Duncan et al., 2014; Rodriguez et al., 2013). Generally, the excitation/inhibition balance is thought to constitute neural activity to a large degree, and the BOLD signal as a surrogate for neural activity is therefore primarily affected by variations in this balance (Duncan et al., 2014; Kapogiannis et al., 2013; Lauritzen et al., 2012; Logothetis, 2008). This important role of the excitation/inhibition balance is not limited to stimulus-induced activity, but also applies to spontaneous activity (Isaacson & Scanziani, 2011). From a developmental perspective, changes from childhood to adulthood in functional connectivity are thus likely associated with concomitant changes in the excitation/inhibition balance, which is closely related to the activities of glutamate and GABA (Duncan et al., 2014). Our finding of the mediating effect of glutamate/H_2O (and thereby GABA+/glutamate) on the decrease of functional connectivity with age supports this hypothesis. However, the precise mechanisms of this effect remain to be elucidated.

4.2.4 Possible mechanisms governing the association between developmental changes in neurotransmitters and in functional connectivity

One likely way of neurotransmitter action on functional connectivity might be synaptic pruning, for which glutamate receptor-mediated plasticity has been shown to be an important factor (Selemon, 2013). Selemon (2013) noted that "the functional significance of synaptic elimination during adolescence, though still enigmatic, probably involves adjustment of the excitatory/inhibitory balance on individual neurons and within networks" (p. 2-3). Evidence shows that there is a relative gain in the inhibitory/excitatory synapse ratio during adolescence and this represents the main argument in support for the functional significance of synaptic pruning (Selemon, 2013). The number of GABAergic inhibitory interneurons remains relatively stable during adolescence, while, in contrast, the number of excitatory connections drops substantially

(Bourgeois & Rakic, 1993; Selemon, 2013; Tau & Peterson, 2009). This fact is most likely reflected in our results of a significant decrease of subcortical glutamate/H_2O and of no significant change in subcortical GABA+/H_2O, resulting in a rise of the GABA+/glutamate ratio. Many other authors discuss the possibility or even the likelihood that developmental functional connectivity changes are associated by some degree with both progressive (e.g. myelination, synapse formation) and regressive (e.g. synaptic pruning) events (Power et al., 2010; Vogel et al., 2010). The reports on the dramatic reduction of synapses occurring during adolescence in the human brain (Huttenlocher & Dabholkar, 1997) make it tempting to interpret these demonstrably concomitant developmental changes as being related, or even that one (i.e. synaptic pruning) might be the cause for the other (i.e. a reduction in functional connectivity with development).

Yet, caution may be warranted when directly linking neurobiological processes, such as synaptic pruning, to descriptive findings, such as changes in functional connectivity since the evidence supporting such interpretations is limited, or in some cases, evidence even seems to refute these interpretations (Paus et al., 2008; Vogel et al., 2010). Vogel et al. (2010) for instance discuss that increases in functional connectivity induced by a modest amount of training in adults are unlikely to be due to myelination or synaptic pruning. Based on the reported changes of functional connectivity due to training, Vogel et al. (2010) propose that developmental changes in networks may be the result of a history of co-activation, as it might be found in Hebbian processes and which is particularly prominent in child and adolescent development. Our results support this notion (even though we do not exclude developmental reductions in glutamate to be related to synaptic pruning as a potential explanation for decreases in functional connectivity). Synchronised (or co-)activity may trigger long term potentiation and consequently synapse maturation and stabilisation (Selemon, 2013). Conversely, a lack of synchronised activity may weaken synaptic strength through long term depression and facilitate synaptic pruning (Selemon, 2013). Both these processes are distinctly dependent on N-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPA) receptors, and both NMDA and AMPA receptors are ionotropic glutamate receptors. Indeed, our result of a positive association between glutamate/H_2O and functional connectivity during development supports this important role of developmental glutamate in the formation of brain networks via co-activation. Thus, we were able to demonstrate for the first time that developmental changes in glutamate/H_2O levels are associated with concomitant changes in functional connectivity and we propose that glutamate is related to functional connectivity due to its role in facilitating synaptic pruning and stabilisation.

4.2.5 Methodological considerations regarding MRS measures

While our results fit nicely to existing theories on putative mechanisms related to developmental changes in functional connectivity, a few methodological aspects of Study B deserve some consideration. On the side of the MRS measures, two points have already been addressed above (i.e. which reference to use for quantification and the low regional specificity of MRS measurements). Further, the currently available MRS methods do not allow to distinguish between the different pools in which both GABA and glutamate are present in the brain (e.g. intra- vs. extracellular levels; Rae, 2013). However, such a distinction or e.g. a classification of "neurotransmitter" vs. "metabolic" glutamate might not be useful in any case because evidence suggests that any pools of both GABA and glutamate can be drawn upon as a source for neurotransmitter GABA and glutamate (Rae, 2013). Arguably the greatest challenge for investigators interested in GABA measurements is the yet unresolved issue of the contamination in the GABA signal by unwanted co-editing macromolecular contributions (Mullins et al., 2014). This issue might be especially relevant for developmental studies since recent evidence suggests that aging might influence the degree of this macromolecular contribution (Aufhaus et al., 2013). While a few approaches have been proposed to deal with this contamination, each of them has some detrimental effects on either acquisition time or data quality (Mullins et al., 2014). Thus, most studies accept the macromolecular contamination as a limitation and reflect this by referring to their GABA levels as GABA+ (as done in Study B). Future studies might be able to use new techniques providing measurements of GABA less affected by this contamination since this challenge represents a field of considerable on-going work (Mullins et al., 2014).

4.2.6 Methodological considerations regarding functional connectivity measures

Functional connectivity analyses are also subject to ongoing controversies and debates. For example, no gold standard has been established with regard to the question which steps should be taken to account for nuisance variables such as breathing and heart rate (Vogel et al., 2010). Further, some studies deem it appropriate to include the regression of the global signal in functional connectivity analyses (e.g. Fox, Zhang, Snyder, & Raichle, 2009), while others advocate that this induces spurious negative correlations (Murphy, Birn, Handwerker, Jones, & Bandettini, 2009). Last, and possibly highly important for developmental studies, functional connectivity analyses might be systematically biased by head motion of participants (Satterthwaite, Wolf, et al., 2013). The findings of Satterthwaite, Wolf, et al. (2013) suggest that age-related connectivity changes are inflated by head motion, specifically the often demonstrated developmental strengthening of long-range connections and the weakening of short-range connections. However, motion seems to impact upon the results in a differential manner: Age-negative correlations seem to be less affected (Chai et al., 2013; Satterthwaite, Wolf, et al., 2013), short-range

connections appear to be less dependent on the choice of processing strategy (Muschelli et al., 2014), and regions near the pivot of the head rotation (e.g. subcortical regions) are the least affected (Satterthwaite, Elliott, et al., 2013). We thus argue that our main findings are valid since our age-related findings were of a negative correlation, short-range, and in a region least affected by motion. Finally, the analysis tool used in Study B (i.e. the conn toolbox; Whitfield-Gabrieli & Nieto-Castanon, 2012) includes the component-based noise correction method aCompCor (Behzadi et al., 2007) and this approach has been shown to effectively deal with motion-related artefacts (Muschelli et al., 2014). Additionally, this approach is thought to yield valid negative correlations since aCompCor does not rely on global signal regression (Whitfield-Gabrieli & Nieto-Castanon, 2012).

Last but not least, the choice of fMRI data that went into the functional connectivity analyses needs to be addressed and thus the remainder of this section is devoted to the discussion of this topic. In Study B, we followed the approach described in Fair, Schlaggar, et al. (2007), which briefly entailed the following: In addition to the usual nuisance regressors included in functional connectivity analyses (i.e. each subject's realignment parameters, and the non-neuronal contributions from WM and cerebrospinal fluid (CSF) by using the aCompCor method (Behzadi et al., 2007) mentioned above), we also included regressors that contained the stimulus onsets and durations of the task as regressors of no interest. The residual timecourses, which were created by removing these modelled effects, were then used for the functional connectivity analyses. This and similar approaches have been used in many recent studies (e.g. R. J. Harris et al., 2014; Norman-Haignere et al., 2012; Zhang et al., 2014). The resulting fMRI data and functional connectivity measures have been referred to in different ways, e.g. "continuous" resting state data (Fair, Schlaggar, et al., 2007), "pseudo-resting state fMRI data" (R. J. Harris et al., 2014), "task residual data" (Zhang et al., 2014), or "background connectivity" (Norman-Haignere et al., 2012) and consequently, the interpretation of the resulting connectivity measures was not completely uniform across these studies.

In our opinion, the term "background connectivity" represents the most appropriate view of data derived in such a fashion. We support the view that such data is characterised by a particular context-dependent brain state. The results of Norman-Haignere et al. (2012) demonstrated that this background connectivity is sensitive to which category is task-relevant, i.e. during face tasks, functional connectivity was selectively found with the face-selective fusiform gyrus, and conversely, with scene-selective parahippocampal cortex during scene tasks (Norman-Haignere et al., 2012). As Norman-Haignere et al. (2012) point out, these findings contradict the view of some researchers who emphasise the consistency of network correlations across rest and task states (e.g. Fox & Raichle, 2007; Fox et al., 2006). These studies suggest that event-related BOLD responses represent a linear superposition of task-related neuronal activity and

ongoing spontaneous activity and that therefore these two types of activity can be separated. In a brilliant review of this topic, Sadaghiani, Hesselmann, Friston, & Kleinschmidt (2010) argue that an accumulating body of evidence is in favour of the hypothesis that ongoing (i.e. spontaneous) brain activity is in fact context-sensitive. They pose the question whether a dissociation of "true intrinsic" (or "pure resting") activity from context-related neural processes is inevitably justified and necessary. The authors instead propose that the function of ongoing activity "is intimately related to cognition, and this relation is inherent to the brain, be it in a 'resting' or active state." (Sadaghiani et al., 2010, p. 4). They acknowledge that functional connectivity networks (ICNs) do show correspondence across very different functional brain states, but they argue it is as true that ICNs resemble spatial patterns with sets of regions that typically show responses in activation studies as a function of the paradigm applied (Smith et al., 2009). In their summary, Sadaghiani et al. (2010) discuss that while the strength of functional connectivity is constrained by structural connectivity, it is importantly modulated by mental states and current context, and they conclude that "intrinsic activity hence constitutes the brain's internal context for processing external information and generating behavior (Fontanini & Katz, 2008; Kenet, Bibitchkov, Tsodyks, Grinvald, & Arieli, 2003)" (Sadaghiani et al., 2010, p. 12).

With regard to our results, we thus interpret our functional connectivity findings as representing intrinsic, yet context-dependent background activity and the context was given by our cognitive task. This may also be the reason why we found a positive correlation between glutatmate/H_2O and local connectivity in the dorsal putamen specifically, since the dorsal part of the putamen (and basal ganglia in general) exhibits more connections to higher cognitive areas such as the dorsolateral prefrontal cortex (Draganski et al., 2008). One could speculate that the use of an emotional task, for instance, would thus result in a positive correlation between glutamate and local connectivity in the ventral putamen, i.e. an area which is more highly connected to cortical areas associated with the processing of emotion, e.g. the orbitofrontal cortex (Draganski et al., 2008). Developmental studies comparing background functional connectivity elicited by different tasks coupled with MRS measures would thus be of high interest.

4.3 Dare we attempt to link it all together?

The findings of Studies A and B provided insight to the relationships between the selected system markers for typical brain development. It is intriguing to imagine going a step further, that is, to link not only two of the markers each but to investigate them all in a comprehensive overall view. The few studies going into this direction are reviewed in Duncan et al. (2014). So far no study has attempted this investigation on a developmental subject group and generally speaking, the amount of knowledge is still limited. For example, studies combining MRS measures and fMRI data exist in adults (e.g. Donahue et al., 2010; Muthukumaraswamy, Edden, Jones, Swettenham, & Singh, 2009; Northoff et al., 2007), but most studies investigated very basic sensory processing (i.e. visual stimulation with checkerboards or simple motor tasks; Duncan et al., 2014). And regarding the relationship between cerebral perfusion and MRS measures, the evidence seems somewhat inconsistent. The correlation between cerebral perfusion and GABA, for instance, has been reported to be positive (Donahue et al., 2010; Michels et al., 2012), negative (Donahue et al., 2014), and other studies yet found no significant relationship (Muthukumaraswamy, Evans, Edden, Wise, & Singh, 2012).

Attempts to link more than two markers were also made within the framework of this thesis, i.e. preliminary analyses were conducted on the triple relationship between neurotransmitter levels, cerebral perfusion, and BOLD responses during our cognitive task. To our surprise, we did not observe any significant correlation between any of the three modalities in our sample of healthy adults. The reasons for this can be manifold and may originate in the theoretical assumptions made or also in the methods chosen. There are, for instance, a dizzying number of methods and tools available to obtain and/or analyse measures from each of the three modalities and each method has its own pitfalls and caveats. The choice of the methods might impact on the successful establishment of relationships between the different measures, especially if the expected relationships are moderate, if the interindividual variability in the data is high, or if the SNR of the measures is low. Regarding the fMRI data available in our project for instance, our task was quite substantially beyond the realm of basic sensory processing (cf. Chapter 3.3.3) that was used in other multimodal studies, and so, the elicited BOLD responses may have been too subtle given the available power (i.e. the number of datasets or subjects). While a more thorough look into this data remains outside the scope of this thesis, this matter surely deserves a deeper investigation.

If prospective studies consider further investigations on this topic, it might be very valuable to additionally include steroid hormone levels in the analyses. Based on our and the findings of many other groups, steroid hormone levels are highly important factors in brain anatomy and physiology in general, and in (pubertal) developmental changes specifically. Moreover, many

studies have provided evidence for interactions between steroid hormones and neurotransmitters such as glutamate and GABA, be it on a cellular level (Zheng, 2009) or in studies with human subjects combining MRS and hormonal measurements (Brawn & Vincent, 2014). To give examples of effects in both directions, fluctuating glutamate levels seemed to affect the rate of production of oestradiol (Balthazart & Ball, 2006), and, in the other direction, oestradiol increased glutamate's binding affinity to receptors (Woolley, Weiland, McEwen, & Schwartzkroin, 1997). Human studies do not yet provide enough information to paint a consistent picture, but a relationship between (sex) steroid hormones and GABA and glutamate is likely since both these neurotransmitters have been reported to vary depending on the menstrual cycle phase (Brawn & Vincent, 2014). In summary, associations have been demonstrated between steroid hormones and virtually the whole range of brain markers measurable with the current MR techniques. Given the dramatic changes in steroid hormone levels in puberty and their probable associations with not only anatomical and physiological brain changes, but also with many psychiatric disorders emerging at this time, further studies linking steroid hormone levels and the range of brain markers available are highly relevant.

Drawing from the experience of the current thesis, studies which plan to tackle this challenge should attempt to attain very high numbers of participating subjects for several reasons. Some associations might be of a small to moderate strength when examined with the relatively macroscopic measures available to non-invasively investigate human subjects (cf. our results on the correlation between oestradiol and testosterone and whole brain perfusion, Chapter 2.4). High statistical power is also important due to the high inter- and intraindividual variability present in most of these measures, and especially so in hormone measures (cf. Chapter 4.1.2). Additionally, if one wants to examine sex differences, the grand total of subjects has to be very large since the group will have to be separated by sex for some analyses, and if the effect of the menstrual cycle phase is of interest, the number of subjects in each female group will again approximately be divided in half. Generally speaking, from our experience, the combination of several measures non-linearly increases complexity of both the recruiting and the measurement aspects of a study. Adolescents wearing a brace on their teeth might be an illustrative example: This will cause severe signal loss in some MR sequences sensitive to susceptibility effects (i.e. fMRI and ASL), while its impact on MRS measures depends on the position of the voxel, and finally, it does not affect hormonal measures at all. Each method is sensitive to different aspects and has its own set of confounding factors and thus, the combination of several methods is accompanied by a great challenge to obtain complete data sets for many subjects due to the heightened possibility of dropouts. While high numbers of participants is beneficial for all studies, they arguably are a mandatory requisite for studies combining different methods.

4.4 Conclusion

This thesis had the aim to investigate the links between major system markers for typical human brain development. Concerning the relationship between steroid hormones and cerebral perfusion, we were able to show that steroid hormones are associated with cerebral perfusion and that DHEAS in particular may be an important factor accounting for the higher perfusion seen in women compared to men. Concerning the developmental changes in the main excitatory and inhibitory neurotransmitters glutamate and GABA, and their associations with concomitant changes in functional connectivity, our findings were twofold: First, we demonstrated an increase in subcortical and cortical GABA+/glutamate with age and second, we found that the developmental decrease in subcortical glutamate/H_2O mediated an age-related decline in local functional connectivity in the dorsal putamen. Taken together, these observations underscore the importance of interactions between major system markers in typical brain development and they provide further evidence that these interactions may contribute to the differential effects the transition from childhood to adulthood has on the human brain as well as on the emergence of many psychiatric disorders.

Abbreviations

AMPA	alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors
ASL	arterial spin labelling
AUC	area under the curve
BOLD	blood oxygenation level-dependent
CBF	cerebral blood flow
CSF	cerebrospinal fluid
CSI	chemical shift imaging
DHEA(S)	refers to both DHEAS and its non-sulphated precursor DHEA
DHEAS	dehydroepiandrosterone sulfate
DTI	diffusion tensor imaging
fMRI	functional MRI
GABA	γ-aminobutyric acid
GM	grey matter
ICA	independent component analysis
ICNs	functional connectivity networks
LC/MSMS	liquid chromatography/tandem mass spectrometry
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
MRSI	magnetic resonance spectroscopic imaging
NAA	N-acetylaspartate
NMDA	N-methyl-D-aspartate
PET	positron emission tomography
RF	radiofrequency
ROI	region of interest
SNR	signal-to-noise ratio
SPECT	single-photon emission computed tomography
WM	white matter

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